

EDUARDO GUATIMOSIM

**MICROBIOTA FITOPATOGÊNICA DAS PLANTAS DANINHAS *Bidens pilosa* E
Bidens subalternans NO BRASIL**

Dissertação apresentada à
Universidade Federal de Viçosa, como
parte das exigências do Programa de
Pós-Graduação em Fitopatologia, para
obtenção do título de *Magister
Scientiae*.

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APROVADA: 21 de Fevereiro de 2011.

Dr. Harold Charles Evans

Prof. Gleiber Quintão Furtado

Prof. Olinto Liparini Pereira
(Presidente da Banca)

À Dada, minha eterna mãe e amiga, dedico.

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BIOGRAFIA

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RESUMO

GUATIMOSIM, Eduardo, M. Sc., Universidade Federal de Viçosa, fevereiro de 2011. **Micobiota fitopatogênica das plantas daninhas *Bidens pilosa* e *Bidens subalternans* no Brasil.** Orientador: Robert Weingart Barreto. Co-Orientadores: Olinto Liparini Pereira e Luiz Antônio Maffia.

O chamado “complexo *Bidens*” é um conjunto de espécies de plantas da família Asteraceae que inclui *Bidens pilosa* (picão preto) e *Bidens subalternans* (picão amarelo). Estas espécies de plantas daninhas são de grande importância devido aos problemas que causam para a agricultura brasileira e mundial. O manejo destas duas espécies tem sido dificultado com o surgimento e disseminação de biótipos resistentes a herbicidas. O controle biológico, já explorado no manejo de outras espécies de plantas daninhas, nunca foi estudado para plantas do “complexo *Bidens*”. Neste trabalho, investigaram-se os fungos associados a estas duas espécies de plantas no Brasil, com vistas a explorar seu potencial de utilização como agentes de controle biológico. Levantamentos foram efetuados num período de vinte e dois meses, incluindo localidades em oito estados brasileiros e resultando na coleta de noventa e sete amostras. Dez espécies fúngicas foram encontradas associadas à *B. pilosa* (**Bp**) e doze associadas a *B. subalternans* (**Bs**): *Colletotrichum* sp. (Bs) (antracnose), *Cercospora bidentis* (Bp e Bs), *Cercospora maculicola* (Bp e Bs) (manchas foliares); *Entyloma bidentis* (Bp e Bs), *Entyloma compositarum* (Bp) e *Entyloma guaraniticum* (Bp e Bs) (carvão branco); *Golovinomyces cichoracearum* var. *cichoracearum* (Bp e Bs) (míldio pulverulento); *Plasmopara halstedii* (Bp e Bs) (míldio verdadeiro); *Pseudocercospora* sp. nov. (Bs) (mancha foliar); *Podospaera fusca* (Bp e Bs) (míldio pulverulento); *Sphaceloma bidentis* (Bs) (verrugose); *Uredo bidentis* (Bp e Bs) e *Uromyces bidentis* (Bp e Bs) (ferrugens). O estudo demonstrou o grande desconhecimento sobre a micobiota destas importantes espécies de plantas invasoras. Todas as associações fungo-hospedeiro, com a exceção de *C. bidentis*, *E. bidentis*, *Uredo bidentis*, e *Uromyces bidentis*, sobre *B. pilosa* e *G. cichoracearum* var. *cichoracearum* e *Uredo bidentis* sobre *B. subalternans* representam relatos novos para o Brasil. Além disto, uma nova espécie (*Pseudocercospora* sp. nov.) foi encontrada e

será proposta. Uma discussão sobre a taxonomia dos fungos coletados é apresentada. Foi comprovada a patogenicidade de *Colletotrichum* sp., *C. bidentis*, *C. maculicola* e *S. bidentis* tanto a *B. pilosa*, quanto a *B. sublaternans*, sendo que apenas *Colletotrichum* sp. e *S. bidentis*, demonstraram potencial aparente como agentes de biocontrole.

ABSTRACT

GUATIMOSIM, Eduardo, M. Sc., Universidade Federal de Viçosa, February of 2011. **Phytopathogenic mycobiota of the weeds *Bidens pilosa* and *Bidens subalternans* in Brazil.** Adviser: Robert Weingart Barreto. Co-advisers: Olinto Liparini Pereira and Luiz Antônio Maffia.

The *Bidens* complex is a set of plant species of the Asteraceae family which includes *Bidens pilosa* and *Bidens subalternans* (Hairy's beggarticks or Spanish needles) two species of weeds with great importance which cause many problems for Brazilian's agriculture and in the world. The management of these species has been difficult with the emergence and spread of herbicide resistant biotypes. Biological control, as explored in the management of other weed species, has never been studied for plants of the *Bidens* complex. In this study, we investigated the fungi associated with these two species in Brazil, with a view to exploring their potential use as biological control agents. Surveys were conducted over a period of twenty-two months, including locations in eight states and resulting in the collection of ninety-seven samples. Ten fungal species were found on *B. pilosa* (Bp) and twelve associated with *B. subalternans* (Bs): *Colletotrichum* sp (Bs) (anthracnose), *Cercospora bidentis* (Bp and Bs), *Cercospora maculicola* (Bp and Bs) (leaf spots); *Entyloma bidentis* (Bp and Bs), *Entyloma compositarum* (Bp) and *Entyloma guaraniticum* (Bp and Bs) (white smuts); *Golovinomyces cichoracearum* var. *cichoracearum* (Bp and Bs) (powdery mildew); *Plasmopara halstedii* (Bp and Bs) (downy mildew); *Pseudocercospora* sp. nov. (Bs) (leaf spot); *Podosphaera fusca* (Bp and Bs) (powdery mildew); *Sphaceloma bidentis* (Bs) (scab); *Uredo bidentis* (Bp and Bs) and *Uromyces bidentis* (Bp and Bs) (rusts). The study demonstrated the widespread ignorance on the mycobiota of these two important species of invasive plants. All fungus-host associations, with the exception of *C. bidentis*, *E. bidentis*, *Uredo bidentis*, and *Uromyces bidentis*, on *B. pilosa* and *G. cichoracearum* var. *cichoracearum* and *Uredo bidentis* on *B. subalternans*, represent new reports for Brazil. Moreover, a new species (*Pseudocercospora* sp. nov.) was found and will be proposed. A discussion on the taxonomy of fungi collected is presented. The pathogenicity of *Colletotrichum* sp., *C. bidentis*, *C. maculicola*, and *S. bidentis* either to *B. pilosa* or to *B. subalternans*, was demonstrated. Only *Colletotrichum* sp. and *S. bidentis* showed potential as biocontrol agents.

INTRODUÇÃO GERAL

As plantas daninhas representam um dos principais fatores limitantes para a produtividade agrícola em todo o mundo. Seu efeito deletério sobre as culturas é múltiplo envolvendo a competição por água, luz ou nutrientes minerais do solo; a interferência na colheita; a contaminação do produto colhido com sementes e outras partes vegetais; o aumento do teor de umidade do produto colhido, dificultando seu beneficiamento e preservação, reduzindo assim o seu valor. Ademais, podem servir de hospedeiro alternativo para pragas e doenças de plantas cultivadas (AULD, 1998).

A preservação da biodiversidade é uma preocupação e desafio mundial, recentemente refletida na escolha, pela ONU, do ano de 2010 como “Ano Internacional da Biodiversidade”. Neste contexto são particularmente oportunos os estudos envolvendo o levantamento e avaliação de componentes negligenciados da biodiversidade, como os fungos, que se destacam como um grupo de organismos megadiversos e pouco conhecidos. São amplamente aceitas as estimativas de que se conheçam menos de 10 % das espécies fúngicas existentes. Esta lacuna de conhecimento tem sido repetidamente confirmada ao longo dos estudos desenvolvidos na UFV sobre fungos fitopatogênicos associados a plantas não cultivadas, particularmente sobre aqueles encontrados atacando plantas daninhas. Estes fungos são ao mesmo tempo pouco conhecidos e potencialmente úteis para o uso no controle biológico de plantas daninhas. Seu estudo pode inclusive, gerar benefícios para o manejo de plantas invasoras de ecossistemas naturais. Plantas estas, apontadas como causadoras de desequilíbrios ambientais devastadores e estando entre as maiores causas da perda de biodiversidade no mundo, depois da destruição dos ecossistemas naturais (CRONK e FULLER, 1995)

O dano econômico causado pelas plantas daninhas, embora reconhecido universalmente como muito expressivo, é de difícil mensuração. As estimativas são vagas e variam para autores e regiões geográficas diferentes. (STEVEN, HOLT e GHERSA, 1997) estimaram que as perdas na produção agrícola mundial seriam de 5% a 10%, enquanto Lorenzi (1982) atribuiu às plantas daninhas uma redução em torno de 20 a 30% da produção agrícola nacional do Brasil. Dentre os métodos de controle

tradicionalmente utilizados para o controle de plantas daninhas, destacam-se os métodos mecânico, químico, cultural e biológico. Pode ser utilizada, também, uma combinação de dois ou mais métodos de controle, conforme as necessidades e condições existentes (ADKINS,1997).

Três espécies de *Bidens* (*B. pilosa*, *B. subalternans*, e *B. alba*) formam o denominado Complexo-*Bidens* no Brasil. *Bidens pilosa* apresenta seis variedades, das quais três ocorrem no Brasil: var. *pilosa*, var. *minor* e var. *radiata*. *Bidens subalternans* também apresenta três variedades ocorrendo no país: var. *subalternans*, var. *simulans* e var. *unipinata*. Características morfológicas são comumente utilizadas para estabelecer relações entre espécies. Entretanto, a grande variação morfológica presente nestas espécies, têm resultado em uma taxonomia imprecisa e controversa ao longo dos anos. Pouco se conhece acerca da origem de *B. pilosa*, sendo o mais aceito é que seu centro de origem seja a América Tropical (Latina e Central). Entretanto esta informação é bastante vaga, pois não há precisão quanto à localidade. A maior parte da informação publicada sobre *Bidens pilosa* se concentra nas reais ou supostas propriedades terapêuticas da planta, focando o seu uso na medicina popular em várias regiões do mundo como anti-inflamatório, antiséptico, regulador de pressão e para o tratamento de hipoglicemia. Há efeitos de hepatoproteção (SUZIGAN, BATTOCHIO, et al., 2009), antimalarial (BRANDÃO, KRETTLI, et al., 1997), e de redução dos níveis de glicose sanguínea (HSU, LEE, et al., 2009) demonstrados para o extrato ou frações de extratos desta planta. Apresenta ainda efeito alelopático inibidor sobre diversas plantas como *Rotala indica*, *Commelina diffusa*, *Jussiaea decurrens*, *Raphanus sativus* e *Echinochloa crus-galli* (KHANH, CONG, et al., 2009), bem como efeito antifúngico in vitro sobre *Corticium rolfsii*, *Fusarium solani* e *Fusarium oxysporum* (DEBA, XUAN, et al., 2008).

O reconhecimento e diferenciação direta das três espécies, principalmente em condições de campo, depende da observação de características morfológicas. *Bidens pilosa*, *Bidens alba* e *Bidens subalternans* apresentam órgãos vegetativos muito semelhantes. *Bidens alba* é perene, ciclo de vida curto, e restrito à regiões costeiras, ao passo que *B. pilosa* e *B. subalternans* são plantas amplamente distribuídas em áreas agrícolas e ao longo das margens de estradas e rodovias. As três espécies apresentam hastes eretas, verdes a salmão; inflorescência tubular de coloração amarela e aquênios estriados de coloração marrom escuro. *Bidens pilosa* apresenta capítulos de duas formas distintas, um de formato radial (com a presença de flores periféricas, sem lígulas

desenvolvidas), e o outro com formato discóide (com a presença de flores centrais); os aquênios apresentam de duas a três aristas. *Bidens alba* apresenta capítulos radiais, com flores periféricas de coloração branca e aquênios com apenas duas aristas. *Bidens subalternans* apresenta capítulos radiais, de coloração amarelo-creme com flores periféricas com lígulas bem desenvolvidas e aquênios com quatro aristas (GROMBONE-GUARATINI, MANSANARES, et al., 2006). Muita controvérsia existe com relação à separação das espécies *B. pilosa*, e *B. subalternans*, Magenta (1998), baseado em características morfológicas, Grombone-Guaratini, Mansanares, et al., (2006), baseado em características bioquímicas e no número distintos de cromossomos ($2n=72$ em *B. pilosa* e $2n=48$ em *B. subalternans*), consideram-nas como duas espécies diferentes. Entretanto, quando comparadas em relação ao padrão de isoenzimas, não foi possível separar uma espécie da outra (GROMBONE-GUARATINI, SEMIR e SOLFERINI, 2005). No presente trabalho serão abordadas apenas as espécies *B. pilosa* e *B. subalternans* e, optou-se por tratá-las como espécies diferentes, por ser esta a opção escolhida pela maioria dos pesquisadores da área, no Brasil. Note-se, no entanto, que provavelmente esta distinção é ora ignorada em outras regiões do mundo e o nome *B. pilosa* é amplamente utilizado para as plantas deste complexo. *Bidens pilosa* está mundialmente dispersa e é hoje considerada pantropical. Trata-se de uma das mais importantes plantas invasoras de culturas no mundo ocorrendo desde áreas agrícolas a jardins e ao longo de rodovias e estradas. Esta espécie é considerada planta daninha no leste da África, Argentina, Austrália, Filipinas, Gana, Havaí, Índia, México, Moçambique, Peru, Suazilândia, Taiwan, Tanzânia, Trindade & Tobago, e Venezuela (HOLM, PLUCKNETT, et al., 1991) e está presente também, em quase todo o território nacional, principalmente nas áreas agrícolas da região Centro-Sul (KISSMANN e GROTH, 1999). *Bidens subalternans* ocorre desde a Argentina e Uruguai à região norte e oeste do Brasil (GROMBONE-GUARATINI *et al.*, 2006). Possivelmente uma distribuição mais ampla, talvez pantropical exista, mas seja mascarada pela omissão de sua distinção em relação a *B. pilosa* em escala mundial. *Bidens pilosa* é uma espécie problemática por muitas razões. É considerada planta daninha de pelo menos 30 culturas em mais de 40 países. É conhecida por reduzir significativamente a produtividade de culturas agrícolas. Na cultura do feijão a colheita foi reduzida em 48% em Uganda e 18 a 48% no Peru. Na cultura do algodão, a presença de aquênios pode contaminar a pluma, bem como a planta impede a cultura de se regenerar devido ao seu efeito aleopático (BIFFE, *et al.*, 2010), além de ser hospedeira alternativa do nematóide

das galhas (*Meloidogyne* sp.) na cultura do algodoeiro (HOLM, PLUCKNETT, et al., 1991). *Bidens pilosa* é também hospedeiro alternativo do vírus do Vira Cabeça do Tomateiro, (*Tomato spotted wilt virus* – TSWV). Muito prolífica e de ciclo curto, é capaz de produzir até três gerações por ano, geralmente formando densas infestações. A formação de sementes é intensa, podendo chegar a 3.000 por planta (ADEGAS *et al.*, 2003). A planta pode gerar densos estandes que não só podem sombrear como também suprimir o crescimento da cultura e da vegetação nativa, devido às suas propriedades alelopáticas. Extratos foliares e de raiz, presentes mesmo após a decomposição da planta, podem suprimir significativamente a germinação e crescimento de mudas de diversas plantas. Além dos prejuízos causados em lavouras, a presença desta planta também pode ocasionar o impedimento ao acesso às estradas bem como trilhas e áreas de lazer. As aristas presentes nos aquênios são um incômodo para as pessoas, bem como ao gado, e ovinos produtores de lã (GISD, 2011).

A eficiência do controle químico para o manejo de *Bidens* spp. no Brasil tem sido comprometida pela ocorrência crescente de resistência aos herbicidas inibidores da enzima acetolactato sintase (ALS) (VIDAL e FLECK, 1997; PONCHIO, 1997, HRAC-BR, 2011). Gelmini (2001), comprovou a existência de biótipos de picão-preto com resistência cruzada aos inibidores da ALS com o grupos das sulfoniluréias e imidazolinonas. A resistência das plantas daninhas aos herbicidas inibidores da ALS é o resultado de uma alteração no gene responsável pela codificação desta enzima (SHANER, 1991). A pressão de seleção gerada pelo uso continuado de herbicidas com um mesmo mecanismo de ação, por sua vez, aumenta a frequência dos biótipos resistentes (RIZZARDI, VIDAL, et al., 2002). Nas lavouras de soja das regiões Centro-Oeste e Sul, tornam-se cada vez mais frequentes relatos de aparecimento de biótipos resistentes de *Bidens* spp. aos inibidores da ALS.

O uso de alternativas para manejar populações de plantas daninhas e invasoras vem crescendo ao longo dos anos. A atenção de muitos pesquisadores tem se voltado para a busca de alternativas ao uso de herbicidas químicos que não ofereçam risco para o meio ambiente, sendo ao mesmo tempo, técnica e economicamente viáveis, dentre estas, destaca-se o controle biológico.

O controle biológico de plantas invasoras por definição é uma estratégia de controle de plantas consideradas problemáticas em um dado ambiente, através do uso de

um ou mais inimigos naturais. Trata-se de um processo seletivo no qual o agente de biocontrole é usado contra determinadas plantas-alvo, sem que haja dano a outras espécies de plantas, sejam elas cultivadas ou nativas da área em questão (GHOSHEH, 2005). Embora existam diversos exemplos do uso de artrópodes em programas de controle biológico, o foco deste trabalho está no uso de fungos como agentes de biocontrole de plantas daninhas. Embora o reconhecimento de que os fitopatógenos (e em particular os fungos) são importantes inimigos naturais de plantas invasoras seja antigo, o seu uso em programas de controle biológico é bastante recente, tendo se iniciado nos anos 70. Diversos autores publicaram revisões completas sobre este tema desde então (HASAN, 1974; HUFFAKER, 1976; HASAN, 1980; TEMPLETON, 1982; WAPSHERE, 1982; TEBEEST, 1984; TEMPLETON, 1984; EVANS, 1987; ADAMS, 1988; AYRES e PAUL, 1990; EVANS e ELLISON, 1990; CHARUDATTAN, 1991; WATSON, 1991; TEBEEST *et al.*, 1992; JULIEN e WHITE, 1997; GHOSHEH, 2005; HALLETT, 2005).

Há duas abordagens para uso de agentes de controle biológico de plantas daninhas: o método clássico, ou inoculativo, o qual envolve a introdução de um inimigo natural hospedeiro-específico, co-evoluído com a planta alvo, desde seu centro de origem até o novo local onde esta, livre de seus inimigos naturais, tornou-se agressiva e o método de bioherbicida ou inundativo, no qual são utilizados fungos tipicamente endêmicos, já associados à planta-alvo, cujo inoculo é produzido em massa e aplicado de modo semelhante a um herbicida químico, em quantidade suficiente para promover o controle da planta daninha. (EVANS, GREAVES e WATSON, 2001; BARRETO, 2004; YANDOC-ABLES, ROSSKOPF e CHARUDATTAN, 2006). Há diversos exemplos de uso bem sucedido e seguro de fungos fitopatogênicos, pelo método clássico, para o controle de plantas invasoras (BARTON, 2004). O presente trabalho trata do estudo de duas espécies de plantas que são importantes como invasoras de ecossistemas agrícolas em regiões consideradas como parte de seu centro de origem e distribuição. Neste caso, a abordagem preferencial é a inundativa ou de bioherbicida.

O método de bioherbicida ou inundativo consiste na produção de propágulos infectivos do agente de biocontrole em larga escala, sendo então aplicados sobre a população da planta-alvo onde é pretendido controlar uma infestação. Esta abordagem tem como premissa o desenvolvimento de formulações e técnicas apropriadas de aplicação, para que um controle adequado seja obtido (TEBEEST, YANG e CISAR,

1992). Alguns bioherbicidas foram desenvolvidos, registrados e/ou comercializados a partir da década de 80 como: COLLEGO® - *Colletotrichum gloeosporioides* (Penz) Sacc. f.sp. *aeschynomene*, para o controle de *Aeschynomene virginica* L.; DEVINE® - *Phytophthora palmivora* (Butler) Butler, para o controle de *Morrenia odorata* (Hook. e Arn.) Lindle; BIOMAL® - *Colletotrichum gloeosporioides* f.sp. *malvae*, para o controle de *Malva pusila* Sm. (*Malva rotundifolia* L.) (FIGUEIREDO, 1995) e CAMPERICO® - *Xanthomonas campestris* pv. *poae*, para o controle de *Poa annua* L. (IMAIZUMI, NISHINO, et al., 1997). Uma lista mais completa, incluindo 12 bioherbicidas foi recentemente publicada (BARRETO, 2009). Muitos outros fitopatógenos estão sendo testados para o desenvolvimento de bioherbicidas em diversos países, como *Drechslera gigantea* (Heald e Wolf) Ito., *Exserohilum longirostratum* (Subram.) Sivan., e *Exserohilum rostratum* (Drechsler) Leonard e Suggs para o controle de *Setaria viridis* (L.) Beauv. na Europa (CASELLA, CHARUDATTAN e VURRO, 2010); *Tobacco mild green mosaic virus* (TMGMV) para o controle de *Solanum viarum* no sul dos Estados Unidos (CHARUDATTAN, 2007); *Alternaria destruens* Simmons (SIMMONS, 1998) para o controle de *Cuscuta pentagona* Engelm. (COOK, CHARUDATTAN, et al., 2009); *Dactylaria higginsii* para o controle de *Cyperus rotundus* L. e *Cyperus esculentus* L. (SHABANA, CHARUDATTAN, et al., 2010). Contudo, observa-se certa frustração quanto às expectativas geradas quando do lançamento de Collego e Devine, há quase trinta anos atrás. Problemas atribuídos à má escolha das plantas-alvo (espécies de plantas daninhas representando pequenos mercados ou que isoladamente não justificariam a existência de um produto específico para seu controle), demasiada especificidade do agente escolhido, dificuldades técnicas na produção massal, formulação e aplicação, combinados ainda com exigências insensatas impostas para o registro de tais produtos, entre outros, frearam o avanço na área. Vários obstáculos têm limitado o interesse da indústria no desenvolvimento de bioherbicidas, entre eles cabe ressaltar: indefinições sobre o papel que o setor público e o privado devem desempenhar no desenvolvimento e comercialização dos produtos, a solução para problemas de metodologia em relação à produção de inóculo, sua aplicação e formulação, e incertezas (ou supostas e muitas vezes exageradas) sobre o risco associado com a utilização desses organismos como agentes de controle, embora nenhum efeito toxicológico adverso tenha sido observado com o uso dos produtos pioneiros COLLEGO e DEVINE (TEBEEEST, 1996). Para que um produto biológico seja usado para o controle de plantas daninhas, ele deve ser fácil de se produzir e

armazenar, de baixo custo, confiável, com altos níveis de controle, de efeito previsível e serem seguros para o ambiente e o homem (TEMPLETON e TEBEEST, 1979). O agente inundativo é cultivado em larga escala, formulado, padronizado, embalado, armazenado e comercializado. Sua comercialização depende de registro prévio e fundamenta-se na periodicidade de aplicação, à semelhança dos herbicidas convencionais (TEBEEST *et al.*, 1992).

Jackson, Schisler, et al., (1996) apontam três métodos para a produção de bioherbicidas: a) uso de plantas hospedeiras in vivo, exclusivo para organismos biotróficos e ainda sem exemplos de viabilidade prática, b) fermentação em substrato sólido, e c) fermentação líquida. A fermentação líquida é o método considerado como o mais viável industrialmente. Para este já existem tecnologias bem conhecidas e equipamentos disponíveis advindos das indústrias de fermentação e têm sido a opção escolhida para a maioria dos produtos que chegaram a ser comercializados (inclusive os pioneiros Collego e Devine).

Dentre os obstáculos ao desenvolvimento de bioherbicidas podemos destacar ainda o estabelecimento de formulações apropriadas. Estas devem proteger os propágulos do microorganismo, aumentar seu crescimento e sobrevivência, bem como mantê-los viáveis e virulentos. A diversidade em relação ao tipo de agente de biocontrole, temperatura e umidade necessária para germinação, infecção, colonização, e esporulação requer que uma variedade de formulações seja desenvolvida (KENNEDY e KREMER, 1996). O maior desafio no desenvolvimento de uma formulação de um bioherbicida é comumente o de favorecer a superação da necessidade que muitos fungos fitopatogênicos têm, de um período de molhamento foliar prolongado. Além disso, com formulações apropriadas pode-se, em princípio, também se obter um bom efeito de controle da planta-alvo com uma dose de inóculo reduzida, melhorando-se o desempenho e reduzindo assim o custo potencial do bioherbicida (AULD e MORIN, 1995). Além disso, algumas estratégias adicionais podem também ser utilizadas para aumentar a eficácia de um bioherbicida. Dentre essas, destaca-se a combinação do agente de biocontrole com herbicidas químicos ou reguladores de crescimento de plantas, aumentando com isso o espectro de ação dos bioherbicidas (CHANDRAMOHAN, CHARUDATTAN, et al., 2000).

O controle biológico clássico de plantas daninhas com fungos fitopatogênicos já é uma estratégia consagrada, embora a sua dependência de financiamento público para ser conduzido e outros problemas alheios à esfera técnico-científica tenham limitado sua utilização. Infelizmente, o mesmo não é válido para a estratégia de bioherbicida. Esta abordagem carece de exemplos mais notáveis de aplicação bem sucedida em grande escala. A busca, pelos pesquisadores na área, pelo desenvolvimento de novos produtos que sejam bem sucedidos técnica e comercialmente tem levado à escolha de plantas-alvo que, isoladamente, causem prejuízos significativos e, ao mesmo tempo, não sejam passíveis de controle eficiente pela aplicação de herbicidas químicos. Esse é o caso de plantas como *B. pilosa* e *B. subalternans*, que causam graves problemas para a agricultura brasileira, tropical e sub-tropical e, ao mesmo tempo, apresenta biótipos resistentes a herbicidas cada vez mais disseminados. As esperanças são de que no futuro, a consolidação dos bioherbicidas como produtos de uso amplo, contribua para significativa redução da contaminação ambiental mundial resultante da aplicação de herbicidas químicos em ambientes agrícolas e outros ecossistemas (BARRETO e EVANS, 1996).

É importante ressaltar que devido à alta similaridade morfológica entre *Bidens pilosa* e *Bidens subalternans*, é possível que grande parte dos fungos descritos em *B. pilosa* se refiram originalmente, a fungos coletados sobre *B. subalternans*, o que pode ser evidenciado pelo número de fungos já descrito nesta última, apenas três, quando comparados às sessenta e três espécies relatadas na primeira (Tab. 1). Trabalhos futuros devem ser realizados, utilizando técnicas moleculares, bem como aproveitando os avanços tecnológicos relacionados à taxonomia clássica, de modo a elucidar a real delimitação tanto das espécies do complexo *Bidens pilosa* quanto dos fungos a estas associadas. No presente trabalho, em todas as coletas o hospedeiro foi cuidadosamente identificado no campo, resultando em informação específica sobre a micobiota que ocorre em cada uma das duas espécies no Brasil.

O estudo aqui desenvolvido é pioneiro para *B. pilosa* e *B. subalternans*. Há poucos registros anteriores publicados de qualquer atividade em controle biológico tendo como alvo estas duas espécies. Em *B. subalternans* há apenas um trabalho publicado envolvendo a busca de agentes de controle biológico, onde somente uma espécie fúngica (*Uromyces bidenticola*) foi considerada com aparente potencial para controle desta planta (DAL BELLO e CARRANZA, 1995). Em *B. pilosa* a única

iniciativa anterior envolveu um levantamento preliminar feito no estado do Rio de Janeiro. Os resultados (não publicados) de Barreto (1991), indicaram a existência de oito espécies de fungos fitopatogênicos associadas a este hospedeiro, sendo que três foram considerados como tendo aparente potencial para uso no controle biológico. Uma lista de fungos potencialmente fitopatogênicos relatados na literatura como associados a *B. pilosa* é também incluída em Barreto (1991) e uma versão atualizada é aqui apresentada (Tab. 1) Trabalhos nas abordagens clássica e inundativa do controle biológico sempre têm como uma de suas etapas iniciais mais importantes a busca e seleção de agentes de controle biológico em potencial. Este trabalho pretende contribuir para atender esta etapa de um programa de longo prazo visando o biocontrole de *B. pilosa* e *B. subalternans*.

Tab 1 – Lista de espécies de fungos já relatados no complexo *Bidens*.

Fungos	Distribuição (Países)	Hospedeiro
Oomycota		
Peronosporales		
<i>Albugo</i> sp.	Venezuela	<i>B. pilosa</i>
<i>Phytophthora nicotianae</i> var. <i>parasitica</i>	EUA, Havaí (EUA)	<i>B. pilosa</i>
<i>Plasmopara halstedii</i>	EUA	<i>B. pilosa</i>
<i>Tetraploa aristata</i>	Cuba, Índias Ocidentais	<i>B. pilosa</i>
Ascomycota		
Botryosphaeriales		
<i>Lasiodiplodia theobromae</i>	Índias Ocidentais, Cuba	<i>B. pilosa</i>
<i>Phyllosticta</i> sp.	Brasil, Cuba, Índias Ocidentais, Venezuela	<i>B. pilosa</i>
Diaporthales		
<i>Diaporthe phaseolorum</i> var. <i>meridionalis</i>	Brasil	<i>B. pilosa</i>
Erysiphales		
<i>Erysiphe lamprocarpa</i>	América do Sul	<i>B. pilosa</i>
<i>Golovinomyces cichoracearum</i> var. <i>cichoracearum</i>	Argentina	<i>B. subalternans</i>
<i>Neoerysiphe cumminsiana</i>	Argentina, Brasil	<i>B. pilosa</i> e <i>B. subalternans</i>
<i>Neoerysiphe galeopsidis</i>	Cuba	<i>B. pilosa</i>
<i>Oidium erysiphoides</i>	América do Sul	<i>B. pilosa</i>
<i>Oidium</i> sp.	Brasil, Cuba, China, Índia, Índias Ocidentais, Jamaica, Malauí, Sudão, Tanzânia, Venezuela, Zimbábue, Zâmbia (IMI 42267)	<i>B. pilosa</i>
<i>Podosphaera fuliginea</i>	África do Sul, China, Índia	<i>B. pilosa</i>
<i>Podosphaera fusca</i>	China, Índia	<i>B. pilosa</i>
<i>Podosphaera macularis</i>	EUA, Ilhas Virgens, Índias Ocidentais, Porto Rico	<i>B. pilosa</i>
Capnodiales		
<i>Cercoseptoria</i> sp.	Índia (IMI 135892)	<i>B. pilosa</i>

<i>Cercospora bidentis</i>	Brasil, cosmopolita (África do Sul, China, Congo, Cuba, EUA, Gana, Guiné, Índia, Índias Ocidentais, Quênia, Laos, Malauí, Malásia, Maurício, Papua Nova Guiné, Samoa, Taiwan, Tailândia, Trindade & Tobago, Venezuela, Zâmbia)	<i>B. pilosa</i>
<i>Cercospora coffeicola</i>	Índia	<i>B. pilosa</i>
<i>Cercospora maculicola</i>	Índia	<i>B. pilosa</i>
<i>Cercospora</i> sp.	Índia (IMI 135892)	<i>B. pilosa</i>
<i>Isariopsis indica</i> var. <i>cynamopsidis</i>	Índia	<i>B. pilosa</i>
<i>Mycosphaerella</i> sp.	Índias Ocidentais, Cuba, Venezuela	<i>B. pilosa</i>
<i>Pseudocercospora megalopotamica</i>	Argentina, China, Colômbia, EUA, Havai (EUA), Uganda	<i>B. pilosa</i>
<i>Septocylindrium concomitans</i>	China	<i>B. pilosa</i>
<i>Septoria balansae</i>	Colômbia	<i>B. pilosa</i>
<i>Cladosporium oxysporum</i>	Índias Ocidentais	<i>B. pilosa</i>
<i>Cladosporium</i> sp.	Quênia (IMI 131266)	<i>B. pilosa</i>
Hypocreales		
<i>Fusarium moniliforme</i>	EUA	<i>B. pilosa</i>
<i>Mycrothecium leuchotrichum</i>	África do Sul (IMI 278126)	<i>B. pilosa</i>
<i>Stachybotrys kampalensis</i>	Índias Ocidentais	<i>B. pilosa</i>
Incertae sedis - Dothideomycetes		
<i>Schiffnerula pulchra</i>	Cuba, Índias Ocidentais	<i>B. pilosa</i>
Incertae sedis - Pezizomycotina		
<i>Gyrothrix</i> sp.	Índias Ocidentais	<i>B. pilosa</i>
Incertae sedis - Sordariomycetes		
<i>Arthrimum</i> sp.	Índias Ocidentais	<i>B. pilosa</i>
<i>Colletotrichum dematium</i>	Índias Ocidentais, Cuba, Venezuela	<i>B. pilosa</i>
<i>Colletotrichum gloeosporioides</i> f. sp. <i>pilosae</i>	Índia	<i>B. pilosa</i>
<i>Colletotrichum</i> sp.	Cuba, EUA, Venezuela	<i>B. pilosa</i>
<i>Glomerella cingulata</i>	Índia, Venezuela (IMI 113592)	<i>B. pilosa</i>
Myriangiales		

<i>Sphaceloma bidentis</i>	Brasil	<i>B. pilosa</i>
<i>Sphaceloma</i> sp.	Zimbábue	<i>B. pilosa</i>
Pleosporales		
<i>Alternaria cucumerina</i> var. <i>cyamopsidis</i>	África do Sul	<i>B. pilosa</i>
<i>Alternaria</i> sp.	Brasil, Venezuela / Zâmbia (IMI 93901)	<i>B. pilosa</i>
<i>Alternaria zinniae</i>	Quênia, África do Sul (IMI 278126)	<i>B. pilosa</i>
<i>Pithomyces bidentis</i>	Taiwan	<i>B. pilosa</i>
<i>Aposphaeria</i> sp.	Índia (IMI 318751)	<i>B. pilosa</i>
<i>Ascochyta</i> sp.	Austrália	<i>B. pilosa</i>
<i>Periconia byssoides</i>	Índias Ocidentais	<i>B. pilosa</i>
<i>Periconia</i> sp.	Índias Ocidentais	<i>B. pilosa</i>
Basidiomycota		
Cantharellales		
<i>Rhizoctonia solani</i>	Brasil	<i>B. pilosa</i>
<i>Thanatephorus cucumeris</i>	Brasil	<i>B. pilosa</i>
Entylomatales		
<i>Entyloma bidentis</i>	Brasil, cosmopolita (África do Sul, Austrália, Bolívia, Colômbia, Costa Rica, Índia, Quênia, Malawi, Malásia, Maurício, Panamá, Papua Nova Guiné, Tailândia, Taiwan, Tanzânia, Zâmbia, Zimbábue)	<i>B. pilosa</i>
<i>Entyloma compositarum</i>	Equador, EUA, Havaí (EUA), Venezuela	<i>B. pilosa</i>

<i>Entyloma guaraniticum</i>	Argentina, cosmopolita (Austrália, China, Costa Rica, Cuba, EUA, Hong-Kong, Índia, Jamaica, Madagascar, Malásia, Malauí, Maurício, Papua Nova Guiné, Paraguai, Porto Rico, Quênia, República Dominicana, Sri-Lanka, Sudão, Tanzânia, Taiwan, Trindade & Tobago, Uganda, Ilhas Virgens, Índias Ocidentais, Zâmbia, Zimbábue)	<i>B. pilosa</i>
<i>Entyloma incertum</i>	Equador, Venezuela	<i>B. pilosa</i>
<i>Entyloma polysporum</i>	EUA, Índias Ocidentais, Venezuela	<i>B. pilosa</i>
<i>Entyloma spegazzinii</i>	África do Sul, Bolívia, Maurício, Tanzânia	<i>B. pilosa</i> & <i>B. subalternans</i>
Sordariales		
<i>Brachysporiella dennisii</i>	Cuba	<i>B. pilosa</i>
<i>Sporidesmiopsis dennisii</i>	Índias Ocidentais	<i>B. pilosa</i>
Trichosphaeriales		
<i>Nigrospora oryzae</i>	Brasil	<i>B. pilosa</i>
Helotiales		
<i>Botrytis cinerea</i>	China	<i>B. pilosa</i>
<i>Sclerotinia libertiana</i>	Taiwan	<i>B. pilosa</i>
<i>Sclerotinia sclerotiorum</i>	África do Sul, Brasil, China	<i>B. pilosa</i>
Pucciniales		
<i>Puccinia obtecta</i>	Cuba, Índias Ocidentais	<i>B. pilosa</i>
<i>Uredo bidentida</i>	Argentina, Paraguai, Uruguai	<i>B. pilosa</i>
<i>Uredo bidenticola</i>	Brasil, Taiwan	<i>B. pilosa</i>
<i>Uredo bidentis</i>	Austrália	<i>B. pilosa</i>
<i>Uredo</i> sp.	Cuba, Índias Ocidentais	<i>B. pilosa</i>

<i>Uromyces bidenticola</i>	Brasil, cosmopolita (Argentina, Austrália, Azores, Bermuda, Bolívia, Brasil, Cabo Verde, Chile, China, Colômbia, Costa Rica, Cuba, Dominica, EUA, Filipinas, Grenada, Guatemala, Haiti, Havai (EUA), Ilhas Canárias, Ilhas Virgens, Índias Ocidentais, Indonésia, Jamaica, Japão, Madagascar, Malásia, Malauí, Maurício, Nigéria, Nova Caledônia, Nova Zelândia, Panamá, Papua Nova Guiné, Porto Rico, Quênia, República Dominicana, Ruanda, Samoa, Sri Lanka, Taiwan, Trindade & Tobago, Uganda, Uruguai, Venezuela, Vietnam, Zimbábue)	<i>B. pilosa</i> e <i>B. subalternans</i>
<i>Uromyces bidenticulata</i>	Índias Ocidentais	<i>B. pilosa</i>
<i>Uromyces bidentis</i>	Brasil, cosmopolita (África do Sul, Colômbia, Equador, EUA, Filipinas, Ilhas Canárias, Ilhas Virgens, Índias Ocidentais, Índia, Jamaica, Nova Zelândia, Porto Rico, República Dominicana, Taiwan, Venezuela)	<i>B. pilosa</i>
<i>Uromyces densus</i>	Porto Rico	<i>B. pilosa</i>
<i>Uromyces martinii</i>	EUA	<i>B. pilosa</i>
<i>Uromyces</i> sp.	Colômbia, Índias Ocidentais	<i>B. pilosa</i>
Urocystidiales		
<i>Thecaphora denticulata</i>	México	<i>B. pilosa</i>
<i>Thecaphora pustulata</i>	Colômbia, Costa Rica, Ilhas Virgens, Índias Ocidentais, Porto Rico	<i>B. pilosa</i>
Incertae sedis		

Incertae sedis		
<i>Sclerotium rolfsii</i>	Zimbábue	<i>B. pilosa</i>

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ARTIGO 1

Artigo elaborado de acordo com as normas da revista Mycopathologia.

The mycobiota of the weeds *Bidens pilosa* and *Bidens subalternans* in Brazil

The mycobiota of the weeds *Bidens pilosa* and *Bidens subalternans* in Brazil

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Abstract

A survey of fungal pathogens associated with two species of the genus *Bidens*: *B. pilosa* and *B. subalternans* (Spanish-needle or Hairy beggarticks) was conducted in Brazil, aimed at finding potential biological control agents for this highly noxious herbaceous neotropical agricultural weed. Ten fungal species were found associated with *B. pilosa* and the following diseases: *Cercospora bidentis* and *Cercospora maculicola* (leaf spots); *Entyloma bidentis*, *Entyloma compositarum* and *Entyloma guaraniticum* (white smuts); *Golovinomyces cichoracearum* var. *cichoracearum* (powdery mildew); *Plasmopara halstedii* (downy mildew); *Podosphaera fusca* (powdery mildew); *Uredo bidentis* and *Uromyces bidentis* (rusts). All fungal species (except *C. bidentis*, *E. bidentis*, *Uredo bidentis*, and *Uromyces bidentis*), represent new records of host-pathogen associations in Brazil. Twelve fungal species were found associated with *B. subalternans* and the following diseases: *Cercospora bidentis* and *Cercospora maculicola* (leaf spots); *Colletotrichum* sp. (anthracnose); *Entyloma bidentis* and *Entyloma guaraniticum* (white smuts); *Golovinomyces cichoracearum* var. *cichoracearum* (powdery mildew); *Plasmopara halstedii* (downy mildew); *Podosphaera fusca* (powdery mildew); *Pseudocercospora* sp.nov. (leaf spot); *Sphaceloma bidentis* (scab); *Uredo bidentis* and *Uromyces bidentis* (rusts). Besides a newly described species (*Pseudocercospora* sp. nov.), all fungal species, except *G. cichoracearum* var. *cichoracearum* and *Uredo bidentis*, represent new host-pathogen associations, confirming the poverty of knowledge on the mycobiota of these highly important weeds. A complete description is provided for each species herein. Pathogenicity was tested for the following selected species: *C. bidentis*, *C. maculicola*, *Colletotrichum* sp., *S. bidentis*, and *Pseudocercospora* sp. nov. A series of methods was attempted for inoculating *E. bidentis*, *E. compositarum* and *E.*

guaraniticum but failed to yield disease symptoms. Typical disease symptoms were obtained after inoculation of *C. bidentis*, *C. maculicola* and *Colletotrichum* sp.

Key words: *Asteraceae*, *Bidens pilosa*, *Bidens subalternans*, mycobiota, biological control, fungal survey.

Introduction

Bidens pilosa and *Bidens subalternans* (Asteraceae), known under common names such as Spanish needle, Hairy's beggarsticks and others (picão preto and picão amarelo in Brazil), are two very similar (morphologically and ecologically) weeds. *Bidens pilosa* has habitats ranging from agricultural areas to disturbed sites, gardens, open waste places, and along roadsides, and occurs as weeds in eastern Africa, Argentina, Australia, Brazil, Ghana, Hawaii, India, Mexico, Mozambique, Peru, Philippines, Swaziland, Taiwan, Tanzania, Trinidad & Tobago, and Venezuela [1]. *Bidens subalternans* occurs from Uruguay and central Argentina to northern and western Brazil [2, 3]. Three species of *Bidens* (*B. pilosa*, *B. subalternans* and *B. alba*) form the *Bidens pilosa* complex in Brazil. *Bidens pilosa* includes six varieties, three of which occur in Brazil: var. *pilosa*, var. *minor* and var. *radiata*, and *B. subalternans* includes three varieties: var. *subalternans*, var. *simulans* and var. *unipinata*. Morphological characters are commonly used to assess relationships among species, but the extensive morphological variation within and among *B. pilosa* populations has resulted in an imprecise and controversial taxonomic characterization of this species. Studies [4] show that most of the voucher specimens identified as *B. pilosa* belong to *B. subalternans*. *Bidens alba* is a perennial, short-lived plant restricted to the coastal areas in Brazil, whereas *B. pilosa* and *B. subalternans* is widely distributed. The three species have green or olivaceous erect, square stems. The disc-floret is yellow and tubular, while the achenes are dark brown and costate. *Bidens pilosa* has two morphs, one radiated (with ray-florets) and the other discoid (without ray-florets). When present, the ray florets vary from white to pink and have achenes with two to three awns. *Bidens alba* has radiated flower heads with white reflexed ray-florets; most achenes have two awns. *Bidens subalternans* has radiated flower

heads with yellow-cream ray-florets; most achenes have four awns [3]. In this study *B. pilosa* and *B. subalternans* are treated as separate species.

Species of the *B. pilosa* complex are problematic for many reasons throughout their range. They infest at least 30 different crops in over 40 countries, are known to significantly reduce crop yields. On dry bean (*Phaseolus vulgaris*), harvests may be reduced by 48% in Uganda and 18-48% in Peru [5]. In agricultural sites cultivated with cotton, the presence of the achenes can contaminate the ball and can have deleterious allelopathic effects [5], and the weeds are known to serve as alternative hosts of the gall-nematode (*Meloidogyne* spp.) [1]. They can form dense stands that can outcompete, outgrow, and eliminate crops and native vegetation, particularly the lower vegetative strata, over large areas. Leaf and root extracts are known to significantly suppress germination and seedling growth of many plants and are believed to remain active throughout decomposition [5]. When forming thick stands they prevent access to roads, trails, and recreational areas. They are also a nuisance to people, as well as sheeps and other fleece producing livestock [5].

In Brazil, the problem can be worsened by the increasingly common occurrence of herbicide resistance for these weeds particularly to acetolactate synthase (ALS) inhibitor herbicides [6, 7, 8]. Biotypes of *B. pilosa* with cross-resistance to herbicides of the sulfonylureas (ALS inhibitors) and imidazolinone groups are known to occur [9]. The failure or increasing costs of herbicide control of such an important agricultural weed encourages investigation of biological control as a potentially sustainable option for its management [10]. There are two basic approaches for weed biological control: the classical or inoculative, and the bioherbicide or inundative. The classical approach is based on the importation and release of highly host-specific exotic natural enemies from the centre of origin or native range of the target weed, whereas the bioherbicide approach is based on the mass production of indigenous pathogens, which may be less host-specific and are found attacking the target-weed in the present area of distribution. Such organisms (usually fungal pathogens) are normally ineffective under natural conditions at controlling weed populations but, after inundative applications of properly formulated inocula on infested area become capable of provoking devastating

epiphytotics that may lead to levels of target weed control equivalent to those attained with chemical herbicide applications [10, 11; 12].

A recent publication accounted for 12 bioherbicides that have been registered and/or marketed worldwide [13]. Most are, nevertheless, small market products targeting weed species of somewhat limited importance. Highly important weed agricultural species have been more intensively targeted lately, as is the present case. Development of such products is commonly preceded by detailed field surveys aimed at yielding the broadest possible list of pathogens adequate for bioherbicide development. This publication documents the results of surveys performed in Brazil of the mycobiota of *B. pilosa* and *B. subalternans*, and also includes detailed information on the taxonomy of each fungal species collected on this host. A preliminary assessment of the pathology and the potential of each fungal species as biological control agents, is also presented

Materials and methods

Survey and sample processing

The survey was performed in Brazil, during 2009-2010, locally in the municipality of Viçosa (state of Minas Gerais), where the research lab is located, and its vicinities, but also included widely distributed collecting sites in Brasília-DF and also in the states of Espírito Santo, Goiás, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo. Roadsides, abandoned areas, agricultural areas, and gardens, where plants of the *B. pilosa*-complex occurred, were searched for seemingly diseased plants. In each site, representative samples were collected. A magnifying 30× hand-lens, was used to select samples in the field. The material was placed and dried in a plant press. Images were taken in the field with a Canon Power-Shot SD890 IS camera. Upon return to the lab, all samples were scrutinized carefully under a dissecting microscope. Hand-free sections of plant tissue containing fungal structures or scraped from colonized plant surfaces were mounted in lactophenol or lactofuchsin. When necessary, sections were prepared with the help of a freezing microtome (Microm HM 520). Observations of fungal structures and measurements, as well as preparation of line

drawing and photographs, were performed with an Olympus BX 51 light microscope fitted with a drawing tube and an Olympus E330 camera. Plates were organized with CorelDraw X4. Representative specimens of the plants were deposited in the herbarium at the Universidade Federal de Viçosa (VIC). Pure cultures were obtained by transfer of spores or other fungal structures, with a sterile fine pointed needle, from lesions onto plates containing PCA (potato carrot-agar). After obtaining pure cultures, these were preserved in silica gel [14], and representative specimens were deposited in the culture collection of the Universidade Federal de Viçosa.

Pathogenicity Tests

Seeds of *B. pilosa* collected in the field in Viçosa – Minas Gerais and Gramado – Rio Grande do Sul, and of *B. subalternans* collected at Visconde do Rio Branco – Minas Gerais and Morro da Fumaça – Rio Grande do Sul were planted in pots (0.5 L) containing soil and manure (1:1) and placed on a greenhouse bench, where they were irrigated regularly, for later use. All the plants used for pathogenicity tests were, at least, 10-15 cm high, and no less than 20 days after germination. Concentration of the inoculum used in each test was calibrated with a Neubauer chamber. All but one of the necrotrophic fungi (*Pseudocercospora* sp.) had their pathogenicity assessed. Details of inoculum production and inoculum application are given below for each group of fungi.

Anthracnose (*Colletotrichum* sp.) – The fungus was grown in plates (60 mm diam) containing PDA (potato dextrose-agar), placed in a growth chamber adjusted to $25 \pm 2^\circ$ C under a light regime of 12 hours (light provided by two fluorescent 40 W General Electric lamps, one F-40 BLB 40 W, spaced 15 cm between each other, placed 35 cm above the plates). Ten mycelial plugs taken from the margin of these cultures were added to 250 mL flasks containing 100 mL of JPB (Jenkins–Prior Broth – [15]). These flasks were left in an orbital shaker at 150 rpm, at $25 \pm 2^\circ$ C, for 15 days. The suspension was filtered through sterile cheese-cloth and the concentration was adjusted to 2.0×10^5 conidia/mL. Two drops of Tween 80, 0.05% were added to the suspension. The final suspension was sprayed onto plants. Plants sprayed with JPB containing two drops of Tween 80, 0.05% served as controls. The plants were left in a moist chamber for 48 hours

and taken to a greenhouse. Each treatment consisted of two repetitions. Evaluation (in the form of symptom observation) was performed 7 days after the inoculation.

Leaf spots (cercosporoids) – Three mycelial plugs were taken from the margin of 20-day old cultures, grown on plates (60 mm diam) containing PDA and added to 250 mL flasks containing 150 mL of PDB (potato dextrose-broth). The flasks were left in an orbital shaker at 150 rpm, at $25 \pm 2^\circ$ C, for 15 days. The fungal biomass was triturated within the liquid medium in an ultraturax and the resulting suspension was poured onto plates (60 mm diam) containing PDA, and left uncovered in a growth chamber adjusted to $25 \pm 2^\circ$ C under a light regime of 12 hours for 72 hours (as above). After the suspension had dried and the plates were fully colonized by the fungus, 10.0 mL of sterile water were poured on each plate and the surface of the sporulating cultures was scraped with a rubber spatula. Two droplets of Tween 20 were added to the conidial suspension, and the concentration was adjusted to 4.0×10^4 conidia/mL (for *C. bidentis*) and 6.0×10^5 conidia/mL (for *C. maculicola*). The conidial suspension was sprayed on the plants until run off. Plants sprayed with sterile water containing two drops of Tween 20 served as controls. The plants were left in a moist chamber for 48 hours and taken to a greenhouse. Each treatment consisted of two repetitions. Evaluations (symptom observations) were performed at 5-day intervals, until the 45th day after the inoculation.

Scab (*Sphaceloma bidentis*) – The fungus was seeded onto plates (60 mm diam) containing PDA and placed in growth chamber adjusted to $25 \pm 2^\circ$ C under a light regime of 12 hours (as above). Ten mycelial plugs were taken from the margin of 20-day old cultures and added to 250 mL flasks containing 150 mL of PDB. These flasks were left in an orbital shaker at 150 rpm, at $25 \pm 2^\circ$ C, for 10 days. The suspension was aseptically filtered in sterile cheese-cloth and 10 ml of the filtered suspension was transferred to flasks containing 100 mL of PDB, and left in an orbital shaker for an additional period of 2 days. The suspension was filtered through sterile cheese-cloth. The concentration of yeast-like cells was adjusted to 2.53×10^7 cells/mL. Two drops of Tween 20 were added to this suspension and it was sprayed onto healthy plants. Separately, the mycelial mass collected on the cheese-cloth was aseptically transferred to a flask containing 100 mL of PDB and was triturated in an ultraturax. The resulting suspension was left on an orbital

shaker at 150 rpm, at $25 \pm 2^\circ \text{C}$, for 2 days. Two drops of Tween 20 were added to the suspension, and the final (non-calibrated) suspension was sprayed onto healthy plants (consisting the second treatment). Plants sprayed with PDB containing two drops of Tween 20 served as controls. Observation of symptoms was made at 5 day intervals, until 22 days after the inoculation.

White smuts (*Entyloma* spp.) – yeast-like cells, sporidia (abundantly produced in culture) and ustilospores were tested separately as inocula of the two species of *Entyloma* that were collected on *B. subalternans*. For the pathogenicity test involving sporidia yeast-like cells of *Entyloma* species, a suspension of yeast cells (1.3×10^6 cells/ml) produced on PCA amended with two drops of Tween 20 was used. It was sprayed until runoff onto healthy *B. subalternans* plants. Plants sprayed with sterile water containing two drops of Tween 20 served as controls. The plants were left in a moist chamber for 48 hours and then taken to a greenhouse. Each treatment consisted of two repetitions. All the plants were observed at 5-day intervals for the appearance of symptoms until 45 days after inoculation. Additionally, injection of such suspensions inside healthy leaves (with a sterile syringe) was also attempted. One further attempt to induce disease symptoms involved immersing *B. pilosa* and *B. subalternans* seeds, in sporidial suspension to try to induce systemic infection of germinating plants, and to test the possible ability of the fungus to affect seed viability of *B. pilosa* and *B. subalternans*. Separate groups of 5 seeds were immersed in an inoculum suspension (1.3×10^6 microconidia/mL) for each of the following periods of time: 0, 1, 10, 15, 30, 45, 60, and 75 minutes, and subsequently cultivated in individual pots (0.5 L), containing sterile (autoclaved) sand. The direct use of ustilospores as inoculum was also tested. Ten fragments (2.0 mm diam) of fresh diseased leaf tissue were crushed in a mortar together with 20 mL of distilled water to yield a suspension of loose ustilospores to be sprayed onto healthy plants. The inoculum concentration was adjusted to 3.5×10^5 ustilospores/mL. Two drops of Tween 20 were added to the resulting suspension, then, the suspension was sprayed onto healthy *B. pilosa* and *B. subalternans* plants. Plants sprayed with sterile water containing two drops of Tween 20 served as controls. The plants were left in a moist chamber for 48 hours and taken to a greenhouse. Each treatment consisted of two repetitions. All the plants were observed at 5-day intervals for disease

symptoms. Finally, the possibility that freshly ejected sporidia directly produced from infected tissues might be needed for infection to occur, was investigated. Ten fragments (2.0 mm diam) of fresh diseased leaf tissue were attached to the inner side of 60 mm diam plates with their abaxial side facing downwards and left on a frame over 10-15 cm tall *Bidens subalternans* plants. This apparatus was mounted within a dew chamber where it was kept for 48 hours and taken to a greenhouse. Each treatment consisted of three repetitions. All the plants were observed at 5-day intervals, no less than 45 days after inoculation.

Germination of ustilospores for observation of morphology

To promote the germination of ustilospores of *Entyloma guaraniticum*, ten fragments (2.0 mm diam) of fresh diseased leaf tissue were crushed in a mortar together with 0.5 mL of distilled water. The final suspension was transferred to a 1.5 mL Eppendorf and left in a growth chamber adjusted to $25 \pm 2^\circ$ C under a light regime of 12 hours, for 24 hours. A drop of the resulting suspension was placed on a slide for observation of possible ustilospore germination under a microscope. As it was conjectured that a dormancy stage might be needed for ustilospore germination, both fresh and older (1, 2, 6, and 11 months old) herbarium samples were examined after that procedure.

Culture description

Anthracnose, leaf spots, and white smuts fungi were isolated by directly aseptically transferring fungal structures with the help of a sterile fine pointed needle onto plates containing PCA. A different procedure was used for isolating the scab fungus. Fresh stem samples showing diseased (scabbed) tissues were vigorously cleaned with pieces of cotton soaked in 90% ethanol and, after evaporation, a flamed scalpel was used to remove a thin superficial layer of epidermis from scabbed tissues and surrounding areas. Then, with the tip of the scalpel, minute amounts of underlying tissue were transferred to plates containing PDA which were placed in growth chamber adjusted to $25 \pm 2^\circ$ C under a light regime of 12 hours, and observed regularly for evidence of colony growth.

Colony descriptions were prepared based on observation of fungal growth on two types of media: PDA and PCA either under a daily 12 hours light regime, or in the

dark (plates wrapped in aluminium foil and kept on the same bench), onto a growth chamber adjusted to $25 \pm 2^\circ$. These were evaluated growth, topography, texture, and color of the colony. Three plates were prepared for each treatment for each fungus. Culture growth was measured and presented as means of colony diameter measured orthogonally at date of description. A standard mycological color chart was used in the description [16].

Results

Fifty-nine samples of diseased *B. pilosa*, and 38 samples of *B. subalternans* were collected during the survey which upon examination yielded ten fungal species on *B. pilosa* and twelve fungal species on *B. subalternans*. Some specimens and cultures were provided by a group based at the Universidade de Patos de Minas (MG). The fungi were associated with the following diseases: anthracnose, downy mildew, leaf spots, powdery mildews, rusts, scab, and white smuts. Descriptions of these fungi are provided below

Anthracnose

Colletotrichum sp.

Teleomorph: *Glomerella* sp. only produced on culture.

Figs. 1, 2, 3

Colonies variable. *Lesions* on stems starting as circular spots, cinamom brown, become discoid, orange, leading to blight, shrivelling and death of infected plants. *Internal mycelium* indistinct. *External mycelium* absent. *Conidiomata* acervular, isolate to crowded, subepidermal, ca 25–125 μm wide, olivaceous brown. *Conidiogenous cells* terminal, often cylindrical, rarely ampuliform, with acute apices, (7.0–)11–20 \times (–24) 2.0–5.0 μm , hyaline. *Conidia* cylindrical, straight to slightly allantoid, apices rounded, (7.0–)9.0–18(–21) \times (3.0–)4.0–5.0(–6.0) μm , aseptate, strongly guttulate hyaline, smooth. *Appressoria* globose to ellipsoid, sometimes irregular, wall one-layered, ca 0.2 μm thick, (6.0–)7.0–10(–12) \times (4.0–)5.0–8.0(–9.0) μm , dark brown to olivaceous brown, smooth.

Material examined: BRAZIL, Goiás, Jataí, road to Rio Verde, on living stems of *B. subalternans*, 13 February 2010, B. S. Vieira (VIC 31566).

Downy mildew

Plasmopara halstedii (Farl.) Berl. & De Toni, in Berlese, De Toni & Fischer, Syll. Fung. (Abellini) 7: 242 (1888)

Fig. 4

Lesions on living leaves, starting as circular to irregular spots, quickly spreading and becoming wide irregular chlorotic to yellowed areas adaxially, accompanied by typical whitish downy areas abaxially, finally coalescing and leading to dark brown blight of foliage. *Internal mycelium* indistinct. *External mycelium* absent. *Sporangiophore* hypophyllous, cylindrical, straight, 6.0–171 × 97–595 µm, base slightly swollen, 8.0–20 µm, monopodially 3–5 branched, mostly at right angles, terminal branches ending in three sterigmata, 13–28 µm, hyaline, aseptate. *Sporangia* terminal, globose to ovoid, 12–26 × 13–19(–22) µm, wall thin, hyaline, smooth.

Material examined: BRAZIL, Minas Gerais, Patos de Minas, BR-365 highway, on living leaves of *B. pilosa*, 11 February 2010, B. S. Vieira (VIC 31535). BRAZIL, Minas Gerais, BR 365, Ituiutaba clover, on living leaves of *B. subalternans*, 12 February 2010, B. S. Vieira (VIC 31563). BRAZIL, Minas Gerais, BR 365 highway, Ituiutaba, on living leaves of *B. subalternans*, 12 February 2010, B. S. Vieira (VIC 31564). BRAZIL, Goiás, São Simão, on living leaves of *B. subalternans* 12 February 2010, B. S. Vieira (VIC 31565). BRAZIL, Minas Gerais, Lagamar, on living leaves and stems of *B. subalternans*, 18 April 2010, B. S. Vieira (VIC 31568). BRAZIL, Minas Gerais, Viçosa, on living leaves of *B. subalternans*, 26 June 2010, E. Guatimosim (VIC 31569). BRAZIL, Goiás, Inacionlândia, road to Itumbiara, on living leaves of *B. pilosa*, 05 December 2010, L. L. Duarte (VIC 31551). BRAZIL, Goiás, Rio Verde, road to Santa Helena, on living leaves of *B. subalternans*, 05 December 2010, L. L. Duarte (VIC 31593). BRAZIL, Minas Gerais, Uberlândia, road to Miraponga, on living leaves of *B. pilosa*, 06 December 2010, L. L. Duarte (VIC 31555). BRAZIL, Minas Gerais,

Uberaba, on living leaves of *B. subalternans*, 06 December 2010, L. L. Duarte (VIC 31597). BRAZIL, Minas Gerais, Patrocínio, road to Ibiá, on living leaves of *B. pilosa*, 07 December 2010, L. L. Duarte (VIC 31549).

Leaf spots

Cercospora bidentis Tharp. Tharp, B.C. Texas parasitic fungi. New species and amended descriptions. *Mycologia* 9 (2): 108 (1917).

Figs. 5, 6

Lesions on living leaves and stems. On leaves starting as minute dark depressed dots becoming circular to irregular necrotic black, centrally raised adaxially elevated, 2.0–9.0 mm diam, coalescing and leading to extensive to complete leaf necrosis. On stems starting as black dots, becoming dry and causing a die-back. *External mycelia* and *stroma* lacking. *Internal mycelium* indistinct. *Conidiophores* arising through the stomata predominantly hypophyllous, rarely epiphyllous, solitary, or forming fascicles of up to 5, sub-cylindrical, straight or slightly curved or sinuose and geniculate, $36\text{--}70 \times 3.5\text{--}6.0 \mu\text{m}$, unbranched, 2–4 septate, pale brown, smooth. *Conidigenous cells* integrated, holoblastic, sub-cylindrical, terminal, sympodial, $8.0\text{--}40 \times 4.5\text{--}6.0 \mu\text{m}$, pale brown. *Conidiogenous loci* 1–5 per cell, discoid, $2.0\text{--}3.0 \mu\text{m}$, strongly thickened, darkened. *Conidia* obclavate to acicular, straight to somewhat curved or slightly sinuous, $55\text{--}172 \times 2.0\text{--}3.5 \mu\text{m}$, apex rounded, base subtruncate, 2–20 septate, strongly guttulate, hyaline, smooth. *Sporulation* abundant, hypophyllous.

In culture: On PDA, slow-growing (2.8–3.0 cm diam after 28 days), moderately elevated centrally, aerial mycelia velvety, olivaceous grey, convoluted on concentric halos, peripheral region of immersed mycelium in short broom-like projections, subtle diurnal zonation; slate blue with brownish reverse; no sporulation. On PCA, slow-growing (4.4–5.0 cm diam after 28 days) flat, aerial mycelia organza-like to powdery, mouse grey to grayish sepia centrally, with petaloid lighter areas irradiating from the center (where mycelium is scarce), grey olivaceous, peripheral region with immersed mycelium in short broom-like

projections grey olivaceous, diurnal zonation pronounced. Dark mouse grey, to greyish sepia, to grey olivaceous reverse; abundant sporulation.

Material examined: BRAZIL, Rio de Janeiro, Murineli, Duas Barras, on living leaves of *B. pilosa*, 02 December 2009, R. W. Barreto (VIC 31542). BRAZIL, Minas Gerais, Viçosa, UFV (Horta Velha), on living leaves of *B. pilosa*, 06 January 2010, E. Guatimosim (VIC 31511). BRAZIL, Minas Gerais, Viçosa, UFV (Horta Nova), on living leaves and stems of *B. pilosa*, 21 January 2010, E. Guatimosim (VIC 31512). BRAZIL, Minas Gerais, Viçosa, UFV (caprinocultura), on living leaves of *B. pilosa*, 25 January 2010, E. Guatimosim (VIC 31513). BRAZIL, Espírito Santo, Belém de Santa Maria, road to Santa Maria de Jetibá, on living leaves of *B. pilosa*, 26 January 2010, R. W. Barreto (VIC 31544). BRAZIL, Minas Gerais, Tiradentes, road to São João Del Rey, on living leaves of *B. pilosa*, 03 February 2010, R. W. Barreto (VIC 31545). BRAZIL, Minas Gerais, Patos de Minas, BR-365 highway, on living leaves of *B. pilosa*, 11 February 2010, E. Guatimosim (VIC 31535). BRAZIL, Minas Gerais, Guimarães, on living leaves and stems of *B. pilosa*, 11 February 2010, E. Guatimosim (VIC 31536). BRAZIL, Minas Gerais, Patos de Minas, on living leaves of *B. pilosa*, 04 March 2010, B. S. Vieira (VIC 31537). BRAZIL, Minas Gerais, Patos de Minas, on living leaves of *B. pilosa*, 04 March 2010, B. S. Vieira (VIC 31538). BRAZIL, Minas Gerais, Patos de Minas, on living leaves of *B. pilosa*, 04 March 2010, B. S. Vieira (VIC 31567). BRAZIL, Minas Gerais, Viçosa, road to Piúna, on living leaves of *B. pilosa*, 02 April 2010, E. Guatimosim (VIC 31514). BRAZIL, São Paulo, Fernão Dias highway, on living leaves of *B. pilosa*, 21 April 2010, E. Guatimosim (VIC 31517). BRAZIL, São Paulo, Guatapará, on living leaves of *B. pilosa*, 22 April 2010, E. Guatimosim (VIC 31518). BRAZIL, Minas Gerais, Viçosa, road to Coimbra, on living leaves and stems of *B. pilosa*, 23 April 2010, E. Guatimosim (VIC 31523). BRAZIL, São Paulo, Mairiporã, next to the Parque Estadual da Cantareira, on living leaves and stems of *B. pilosa*, 24 April 2010, E. Guatimosim (VIC 31515). BRAZIL, São Paulo, Mairiporã, next to the Parque Estadual da Cantareira, on living leaves and stems of *B. pilosa*, 24 April 2010, E. Guatimosim (VIC 31519). BRAZIL, Rio Grande do Sul, Taquara, road to Santo Antunes, on living leaves of *B. pilosa*, 16 October 2010, E. Guatimosim (VIC 31526). BRAZIL, Santa Catarina, Morro da Fumaça, road to Cocal do Sul, on living

leaves and stems of *B. pilosa*, 17 October 2010, E. Guatimosim (VIC 31528). BRAZIL, São Paulo, Itatiba, on living leaves and stems of *B. pilosa*, 19 October 2010, E. Guatimosim (VIC 31532). BRAZIL, Goiás, Pantaninho, on living leaves of *B. pilosa*, 06 December 2010, L. L. Duarte (VIC 31557). BRAZIL, Minas Gerais, Ibiá, on living leaves of *B. pilosa*, 07 December 2010, L. L. Duarte (VIC 31547). BRAZIL, Minas Gerais, Patrocínio, road to Ibiá, on living leaves of *B. pilosa*, 07 December 2010, L. L. Duarte (VIC 31548). BRAZIL, Minas Gerais, Patrocínio, road to Ibiá, on living leaves of *B. pilosa*, 07 December 2010, L. L. Duarte (VIC 31550). BRAZIL, Minas Gerais, Viçosa, on living leaves and stems of *B. subalternans*, 26 June 2010, E. Guatimosim (VIC 31569). BRAZIL, Paraná, Mamborê, road to Campo Mourão, on living leaves of *B. subalternans*, 13 October 2010, E. Guatimosim (VIC 31571). BRAZIL, Paraná, Concordia, after to the gas station Castelo, on living leaves of *B. subalternans*, 15 October 2010, E. Guatimosim (VIC 31581). BRAZIL, Rio Grande do Sul, Santa Teresa, near to graveyard, on living leaves of *B. subalternans*, 16 October 2010, E. Guatimosim (VIC 31585). BRAZIL, Minas Gerais, Patos de Minas, on living leaves and stems of *B. subalternans*, 15 October 2010, B. S. Vieira (VIC 31567). BRAZIL, São Paulo, Jiquiá, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31587). BRAZIL, Santa Catarina, Pomerode, in front of the drugstore Najufarma, on living leaves and stems of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31588). BRAZIL, Santa Catarina, Morro da Fumaça, road to Coal do Sul, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31583). BRAZIL, Santa Catarina, Cocal do Sul, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31586). BRAZIL, Santa Catarina, Braço do Norte, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31582). BRAZIL, Santa Catarina, Morro da Fumaça, road to Urussanga, on living leaves and stems of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31584). BRAZIL, Santa Catarina, Guaramirim, road to Joinville, on living leaves of *B. subalternans*, 18 October 2010, E. Guatimosim (VIC 31577). BRAZIL, Santa Catarina, Jaguará do Sul, on living leaves of *B. subalternans*, 18 October 2010, E. Guatimosim (VIC 31579). BRAZIL, Goiás, Jataí, road to Rio Verde, on living leaves of *B. subalternans*, 12 December 2010, L. L. Duarte (VIC 31594).

Cercospora maculicola Thirum. & Govindu. Notes on some Indian Cercosporae
V. Sydowia 8(1-6): 344 (1954)

Figs. 7, 8

Lesions on living leaves and stems: on leaves, leaf spots amphigenous starting as minute dark depressed dots becoming circular to irregular, necrotic, dark, centrally and slightly protuberant with elevated margins bearing some grayish dark, 1–9 mm diam; on stems starting as black dots, becoming dry and causing a die-back. *Internal mycelia* septate, intercellular, 4.0–5.0 μm diam, rarely branched, sub-hyaline. *External mycelium* absent. *Stromata* absent. *Conidiophores* raising through the stomata predominantly hypophyllous, rarely epiphyllous, solitary, or forming rudimentary fascicles (of up to 5 conidiophores), sub-cylindrical, geniculate, 24–64 \times 4.0–6.5 μm , restricted to the conidiogenous cell, non-septate or rarely 1-septate at the base, unbranched, sub-hyaline to light olivaceous brown, smooth. *Conidigenous cells* integrated terminal, sub-cylindrical sympodial, 9.0–19 \times 5.0–6.5 μm , sub-hyaline. *Conidigenous loci* conspicuous, 1–3 per cell, 2.0–2.5 μm , strongly thickened, darkened. *Conidia* obclavate to cylindrical, straight to slightly curved, 45–99 \times 2.5–4 μm , rounded apex, obtruncate base, 1–10 septate, guttulate, smooth.

In culture: On PCA, slow-growing (6.0 cm diam, after 28 days), circular, flat, predominantly immersed, pigmenting the medium in rose, aerial mycelium scarce, cottony centrally, followed by an area with sparse mycelia, followed by a narrow ring of dense mycelium, followed by a periphery of powdery mycelium, white mixed with pale mouse grey, diurnal zonation evident; rose intermixed with grey sepia reverse, no sporulation. On PDA, slow-growing (5.3–5.6 cm diam after 28 days), circular, with lobed edges, slightly elevated centrally, white with some orange pigmentation, somewhat furrowed radially and partly circular compressed and raised concentric areas, presence of white spots of suedly aerial mycelium, followed by a zone of felty mouse grey aerial mycelium, and another zone of suedly iron grey aerial mycelium and, periphery of immersed mycelium in short bristles projections. Greyish blue to brownish reverse; no sporulation.

Material examined: BRAZIL, Minas Gerais, Viçosa, UFV (Gado de Leite), on living leaves and stems of *B. pilosa*, 15 August 2009, E. Guatimosim (VIC 31506). BRAZIL, Minas Gerais, Itabirito, road to São Gonçalo do Bação, on living leaves of *B. pilosa*, 07 January 2010, E. Guatimosim (VIC 31509). BRAZIL, Minas Gerais, São Gonçalo do Abaeté, on living leaves and stems of *B. pilosa*, 18 April 2010, B. S. Vieira (VIC 31541). BRAZIL, Minas Gerais, Patos de Minas, on living leaves and stems of *B. pilosa*, 18 April 2010, B. S. Vieira (VIC 31539). BRAZIL, Minas Gerais, Tiradentes, road to São João Del Rey, on living leaves and stems of *B. pilosa*, 21 April 2010, E. Guatimosim (VIC 31516). BRAZIL, Minas Gerais, Viçosa, on living leaves of *B. subalternans*, 26 June 2010, E. Guatimosim (VIC 31569). BRAZIL, Paraná, Capivari, on living leaves of *B. subalternans*, 12 October 2010, E. Guatimosim (VIC 31590). BRAZIL, São Paulo, Sorocaba, on living leaves of *B. pilosa*, 19 October 2010, E. Guatimosim (VIC 31533). BRAZIL, São Paulo, Itatiba, on living leaves of *B. subalternans*, 19 October 2010, E. Guatimosim (VIC 31591). BRAZIL, Goiás, Inacionlândia, road to Itumbiara, on living leaves of *B. subalternans*, 05 December 2010, L. L. Duarte (VIC 31592).

Pseudocercospora **sp. nov.**

Figs. 9, 10

Leaf spots irregular shaped, dark brown to dark green when dry, becoming vein-delimited, coalescing and expanding towards the leaf edges, 5.0–35.0 × 4.0–15.0 mm, leading to entire leaf blight. *Internal mycelium* intercellular, 2.0–3.0 µm diam, strongly branched, septate, hyaline. *External mycelium* lacking. *Stromata* substomatal, globose, 25–64 µm, subhyaline to slightly brownish olivaceous. *Conidiophores* with caespituli amphigenous, raising above the stroma through stomata, in loose fascicles of numerous conidiophores, subcylindrical, curved or sinuous, geniculated at the apex, 10–59 × 2.0–3.0 µm, (0–)1–4 septate, rarely branched, hyaline, smooth. *Conidiogenous loci* inconspicuous. *Conidia* solitary, acicular, 40–77 × 1.0–2.0 µm, sub-acute to rounded apex, obconic base, 2–8

septate, conidial scars inconspicuous, strongly guttulate, medium to highly sinuose, hyaline, smooth.

In culture: On PCA, slow-growing (4.2–4.6 cm diam after 42 days), circular, flat, aerial mycelium scarce felty, centrally pale mouse grey to olivaceous grey, with white spots of aerial mycelium; presence of areas with aerial mycelium absent, periphery strongly composed of immersed mycelium in short bristles projections, dark herbage green; diurnal zonation absent; humidity slightly present centrally; leaden black to olivaceous black reverse; no sporulation. On PDA, very slow-growing (1.3–1.7 cm diam after 42 days), circular, with lobed edges, slightly elevated, pigmenting the media to grayish rose, aerial mycelium suedy olivaceous grey centrally, and iron grey edges, presence of white spots of cottony aerial mycelium, centrally spread and, narrow periphery of immersed mycelium in short bristles projections olivaceous black; diurnal zonation absent, humidity absent. Slate blue reverse; no sporulation.

Host range distribution: only known from the type collections.

Examined materials: BRAZIL, Minas Gerais, BR 365, Ituiutaba clover, on living leaves of *B. subalternans*, 12 February 2010, B. S. Vieira (VIC 31563). BRAZIL, Minas Gerais, BR 365 highway, Ituiutaba, on living leaves of *B. subalternans*, 12 February 2010, B. S. Vieira (VIC 31564). BRAZIL, Paraná, Lorenzal, on living leaves of *B. subalternans*, 13 October 2010, E. Guatimosim (VIC 31574). BRAZIL, Paraná, Lindoeste, road to Capitão Leonidas Marques, on living leaves of *B. subalternans*, 14 October 2010, E. Guatimosim (VIC 31576). BRAZIL, Santa Catarina, Morro da Fumaça, road to Coal do Sul, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31583). BRAZIL, Goiás, Cachoeira Dourada, on living leaves of *B. subalternans*, 05 December 2010, L. L. Duarte (VIC 31596). BRAZIL, Minas Gerais, Uberaba, on living leaves of *B. subalternans*, 06 December 2010, L. L. Duarte (VIC 31599).

Powdery mildews

Golovinomyces cichoracearum var. *cichoracearum* (DC) V.P. Heluta, Ukr. Bot. Zh. 45(5): 62 (1988)

Colonies on living leaves and stems, starting as imperceptible mycelial growth on leaves and stems that become scarce and hard visible powdery white masses on leaves and stems, but mainly on leaves, fungal growth without evident changes on underlying tissues until later stages when colonies coalesce and infected plants are led to premature death. *External mycelia* straight to flexuous, (4.0–)5.0–7.0(–7.5) μm diam, sparingly branched, septate, hyaline, smooth, persistent. *Conidiophores* erect, straight, cylindrical, (43–)55–90(–114) \times 10–13 μm , 1–4 septate. *Foot cells* straight, cylindrical, constricted at the basal septum, (40–)43–90(–114) μm long, 10–12(–13) μm wide, followed by 1–3 cells, basal septum (7.0–)9.0–10 μm . *Mycelial appressoria* nipple-shaped, sometimes lobed. *Conidia* in short chains, 1–3 conidia per chain, ovoid-ellipsoid to doliform-cylindrical, with fibrosin bodies, (23–)27–30(–34) \times 12–19 μm , l/w ratio 1.91, aseptate, germinating at the apex, hyaline, smooth. *Teleomorph* absent.

Material examined: BRAZIL, Paraná, Chapecó, road to Concórdia, on living leaves and stems of *B. pilosa*, 15 October 2010, E. Guatimosim (VIC 31527). BRAZIL, Rio Grande do Sul, Bento Gonçalves (vale dos Vinhedos), on living leaves and stems of *B. pilosa*, 16 October 2010, E. Guatimosim (VIC 31530). BRAZIL, Paraná, Guarapuava, road to Turvo, on living leaves and stems of *B. subalternans*, 12 October 2010, E. Guatimosim (VIC 31573). BRAZIL, Paraná, Ponta Grossa, road to Guarapuava, on living leaves and stems of *B. subalternans*, 12 October 2010, E. Guatimosim (VIC 31572). BRAZIL, Paraná, Mamborê, road to Campo Mourão, on living leaves and stems of *B. subalternans*, 13 October 2010, E. Guatimosim (VIC 31571). BRAZIL, Paraná, Altamira do Paraná, road to Nova Cantu, on living leaves and stems of *B. subalternans*, 13 October 2010, E. Guatimosim (VIC 31589). BRAZIL, Paraná, Salto do Lontra, road to Realeza, on living leaves and stems of *B. subalternans*, 14 October 2010, E. Guatimosim (VIC 31575). BRAZIL, Paraná, Lindoeste, road to Capitão Leonidas Marques, on living leaves of *B. subalternans*, 14 October 2010, E. Guatimosim (VIC 31576). BRAZIL, Paraná, Concórdia, on living leaves of *B. subalternans*, 15 October 2010, E. Guatimosim (VIC 31581). BRAZIL, Rio Grande do Sul, Nova Petrópolis, on living leaves of *B. subalternans*, 16 October 2010, E. Guatimosim (VIC 31580). BRAZIL, São Paulo, Juquiá, on living leaves of *B. subalternans*, 17

October 2010, E. Guatimosim (VIC 31587). BRAZIL, Santa Catarina, Guarimirim, road to Joinvile, on living leaves and stems of *B. subalternans*, 18 October 2010, E. Guatimosim (VIC 31577). BRAZIL, Santa Catarina, Joinvile, on living leaves and stems of *B. subalternans*, 18 October 2010, E. Guatimosim (VIC 31578).

Podosphaera fusca (Fr.) U. Braun & Shishkoff, in Braun & Takamatsu, Schlechtendalia 4: 29 (2000)

Fig. 12

Colonies on living leaves and stems, starting as imperceptible mycelial growth on leaves and stems that become denser and visible in time as circular to sub-circular powdery white masses amphigenous on leaves and on stems, fungal growth without evident changes on underlying tissues until later stages when colonies coalesce and infected plants become chlorotic leading to premature death. *External hyphae* straight to flexuous, 5.0–8.0 µm diam, unbranched, septate, hyaline, smooth, persistent. *Mycelial appressoria* often lobed, sometimes slightly nipple-shaped. *Internal hyphae* absent. *Conidiophores* erect, straight, cylindrical to clavate, (41–)63–95(–126) × 11–16 µm, 1–6 septate. *Foot cells* straight, cylindrical, 40–126 × 11–16 µm, basal septum (8.0–)9.0–10(–11) µm displaced from the branching point of the mycelium. *Conidia* in short chains, 2–3 conidia per chain, ovoid-ellipsoid to doliform, with fibrosin bodies, 22–35 × 13–19 µm, l/w ratio 1.80, aseptate, germ tubes short (shorter than conidial length) mostly oriented laterally without producing appressoria. *Teleomorph*: absent.

Material examined: BRAZIL, Minas Gerais, Viçosa, UFV (Clínica de Doenças de Plantas), on living leaves of *B. pilosa*, 15 August 2009, E. Guatimosim (VIC 31504). BRAZIL, Minas Gerais, Viçosa, UFV (Gado de Leite), on living leaves and stems of *B. pilosa*, 15 August 2009, E. Guatimosim (VIC 31506). BRAZIL, Minas Gerais, Viçosa, UFV (Clínica de Doenças de Plantas), on living leaves of *B. pilosa*, 01 September 2009, E. Guatimosim (VIC 31505). BRAZIL, Minas Gerais, Viçosa, UFV (Fruticultura), on living leaves and stems of *B. pilosa*, 24 September 2009, E. Guatimosim (VIC 31507). BRAZIL, Minas Gerais, Itabirito,

road to São Gonçalo do Bação, on living leaves of *B. pilosa*, 07 January 2010, E. Guatimosim (VIC 31509). BRAZIL, Minas Gerais, Viçosa, UFV (Horta Nova), on living leaves and stems of *B. pilosa*, 21 January 2010, E. Guatimosim (VIC 31512). BRAZIL, Minas Gerais, Tiradentes, road to São João Del Rey, on living leaves of *B. pilosa*, 03 February 2010, R. W. Barreto (VIC 31545). BRAZIL, Minas Gerais, Patos de Minas, BR-365 highway, on living leaves of *B. pilosa*, 11 February 2010, B.S. Vieira (VIC 31535). BRAZIL, Minas Gerais, BR 365, Ituiutaba clover on living leaves and stems of *B. subalternans*, 12 February 2010, B. S. Vieira (VIC 31563). BRAZIL, Minas Gerais, Lagamar, on living leaves and stems of *B. subalternans*, 18 April 2010, B. S. Vieira (VIC 31568).

Rusts

Uredo bidentis Henn., Hedwigia 35: 251 (1896) Teleomorph *Uromyces bidenticola* Arthur. Uredinales of Porto Rico based on collections by H.H. Whetzel and E.W. Olive. Mycologia 9(2): 71 (1917)

Figs. 13, 14

Lesion on leaves showing no symptoms, pustules in dispersed groups, slightly elevated, erumpent, amphigenous. *Uredinia* amphigenous, but more crowded abaxially, erumpent. *Urediniospores* ovoid, ellipsoid to subglobose, 28–30 × 22–25 µm, orange to cinnamon brown, wall 1-layered, thickened, 2.0–3.0 µm, echinulate except over the pores. *Pores* indistinct (but described as two equatorial or supra-equatorial in the literature). *Telia*, *Aecial* and *Spermogonial* stages not seen.

Material examined: BRAZIL, Minas Gerais, Fervedouro, road to Araponga, on living leaves of *B. pilosa*, 27 July 2009, E. Guatimosim (VIC 31508). BRAZIL, Minas Gerais, Viçosa, UFV (Horta Nova), on living leaves of *B. pilosa*, 21 January 2010, E. Guatimosim (VIC 31512). BRAZIL, Minas Gerais, Patos de Minas, BR-365 highway, on living leaves of *B. pilosa*, 11 February 2010, B. S. Vieira (VIC 31535). BRAZIL, Rio Grande do Sul, Bento Gonçalves (vale dos Vinhedos), on living leaves of *B. pilosa*, 16 October 2010, E. Guatimosim (VIC 31530). BRAZIL, Santa Catarina, São Francisco do Itaperiú, road to

Massaranduba, on living leaves of *B. pilosa*, 18 October 2010, E. Guatimosim (VIC 31525). BRAZIL, São Paulo, Itatiba, on living leaves of *B. pilosa*, 19 October 2010, E. Guatimosim (VIC 31532). BRAZIL, São Paulo, Morungaba, road to Amparo, on living leaves of *B. pilosa*, 19 October 2010, E. Guatimosim (VIC 31534). BRAZIL, Paraná, Ponta Grossa, road to Guarapuava, on living leaves of *B. subalternans*, 12 October 2010, E. Guatimosim (VIC 31572). BRAZIL, Paraná, Lindoeste, road to Capitão Leonidas Marques, on living leaves of *B. subalternans*, 14 October 2010, E. Guatimosim (VIC 31576). BRAZIL, Rio Grande do Sul, Nova Petrópolis, on living leaves of *B. subalternans*, 16 October 2010, E. Guatimosim (VIC 31580). BRAZIL, Rio Grande do Sul, Santa Teresa, near to graveyard, on living leaves of *B. subalternans*, 16 October 2010, E. Guatimosim (VIC 31585). BRAZIL, São Paulo, Juquiá, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31587). BRAZIL, Santa Catarina, Cocal do Sul, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31586). BRAZIL, Santa Catarina, Joinville, on living leaves of *B. subalternans*, 18 October 2010, E. Guatimosim (VIC 31578). BRAZIL, Santa Catarina, Jaguará do Sul, on living leaves of *B. subalternans*, 18 October 2010, E. Guatimosim (VIC 31579).

Uromyces bidentis Lagerh., Lagerheim, in Patouillard & Lagerheim, Bull. Soc. Myc. France 11: 213. 1895.

Figs. 15, 16

Lesion on leaves showing no symptoms adaxially, pustules in close groups, slightly elevated, erumpent abaxially. *Spermogonia*, *aecia* and *uredinia* lacking. *Telia* hypophyllous in close, groups. *Teliospores* oblong to ellipsoid or elongate to obovoid, $32\text{--}41 \times 15\text{--}20 \mu\text{m}$, cinnamon brown, wall 1-layered, *ca* $1.0 \mu\text{m}$ thick laterally, umbo subhyaline, $6.0\text{--}8.0 \mu\text{m}$ long, smooth. *Pedicel* $60\text{--}72 \times 3.0\text{--}4.0 \mu\text{m}$, hyaline. *Teliospores germinating* by formation of promycelium, septate, guttulate, hyaline, smooth, with four basidiospores hyaline, smooth. *Teliospore* becoming paler and grayish after germination.

Material examined: BRAZIL, Minas Gerais, Viçosa, UFV (Clínica de Doenças de Plantas), on living leaves of *B. pilosa*, 15 August 2009, E. Guatimosim (VIC 31504). BRAZIL, Minas Gerais, Viçosa, UFV (Gado de Leite), on living leaves of *B. pilosa*, 15 August 2009, E. Guatimosim (VIC 31506). BRAZIL, Minas Gerais, Viçosa, UFV (Fruticultura), on living leaves of *B. pilosa*, 24 September 2009, E. Guatimosim (VIC 31507). BRAZIL, Rio Grande do Sul, Gramado, on living leaves of *B. pilosa*, 16 August 2010, E. Guatimosim (VIC 31529). BRAZIL, Rio Grande do Sul, Santa Teresa, road to Gramado, on living leaves of *B. pilosa*, 16 August 2010, E. Guatimosim (VIC 31531). BRAZIL, Paraná, Altamira do Paraná, road to Nova Cantu, on living leaves of *B. subalternans*, 13 October 2010, E. Guatimosim (VIC 31589).

Scab

Sphaceloma bidentis Bitanc. & Jenkins emend. descr. Estudos Sobre as Miriangiales. Archos Inst. Biol., S. Paulo 20: 5 (1951)

Figs. 17, 18, 19, 20

Lesions on leaves and stems: on leaves, mostly along secondary veins, hypophyllous, depressed, irregular, $0.4\text{--}2.2 \text{ mm}$ diam, leading to disintegration

and flecking of host tissue and only epidermal cells remaining, white centered; on stems, typical scab symptoms with numerous rounded to irregular warts, with russet vinaceous sorghum brown haloes, and vinaceous buff to avellaneous centre, slightly wrinkly. Depending on intensity, leading to distortions of growing stems that may become sinuous or twisted and accompanied by defoliation and die-back of above infected areas. *Internal mycelium* septate, branched in acute angles, 2–3 µm diam, with some enlarged rounded cells, hyaline, smooth, often producing chlamydospores. *Acervuli* almost indistinct, erumpent, localized over a hyaline pseudoparenchyma, formed by 2–3 layers of swollen, irregular cells, 30–100 µm long. *Conidiogenous cells* ampulliform, with an acute apex, 7.0 µm, hyaline, smooth. *Conidia* sub-cylindrical, 3.0–5.0(–8.0) × 2.0–4.0 µm, hyaline, smooth.

In culture: On PCA, very slow-growing (1.3–1.6 cm, after 30 days) circular, compressing the medium. Aerial mycelium cottony, forming a pinkish white subiculum. Immersed mycelia forming a distinct livid red feathery periphery. Dark vinaceous with a distinctly feathery periphery reverse; sporulation absent.

Examined materials: BRAZIL, Paraná, Lorenzal, on living leaves of *B. subalternans*, 13 October 2010, E. Guatimosim (VIC 31574). BRAZIL, Rio Grande do Sul, Nova Petrópolis, on living stems of *B. subalternans*, 16 October 2010, E. Guatimosim (VIC 31580).

White smuts

Entyloma bidentis Henn., Ustilaginaceae. Brandpilze. Pflanzenw. Ost-Afrikas Nachbarg., Teil C.: 49 (1895)

Fig. 21

Lesions on living leaves, starting as minute chlorotic dots, becoming circular and flat, 1.0–5.0 mm diam, yellow-greenish, becoming dry and necrotic with expanding and leading to leaf blight, and death. *Sori* of densely packed and embedded in host tissue. *Internal mycelium* not seen. *Ustilospores* subglobose to ellipsoid, 11–15(–18) × 12–15 µm, subhyaline to yellowish-brown, wall two-layered, the inner layer thin, regular, ca 1.0 µm, the outer slightly thick, regular,

1.0–2.0 μm , smooth. *Anamorph* arising through stomata, sickle-shaped; sporidia up to $45\text{--}50 \times 1.0\text{--}2.0 \mu\text{m}$, strongly guttulate, hyaline, smooth, needle-shaped sporidia not seen.

Material examined: BRAZIL, Rio de Janeiro, Murineli, Duas Barras, on living leaves of *B. pilosa*, 02 December 2009, R. W. Barreto (VIC 31542). BRAZIL, Minas Gerais, Viçosa, UFV (Horta Velha), on living leaves of *B. pilosa*, 06 January 2010, E. Guatimosim (VIC 31511). BRAZIL, Minas Gerais, Viçosa, UFV (Horta Nova), on living leaves and stems of *B. pilosa*, 21 January 2010, E. Guatimosim (VIC 31512). BRAZIL, São Paulo, Piracicaba, road to Limeira, on living leaves of *B. pilosa*, 23 April 2010, E. Guatimosim (VIC 31521). BRAZIL, Rio Grande do Sul, Santa Teresa, road to Gramado, on living leaves of *B. pilosa*, 16 October 2010, E. Guatimosim (VIC 31531). BRAZIL, Santa Catarina, São Francisco do Itaperiú, road to Massaranduba, on living leaves of *B. pilosa*, 18 October 2010, E. Guatimosim (VIC 31525). BRAZIL, Goiás, Pantaninho, on living leaves of *B. pilosa*, 06 December 2010, L. L. Duarte (VIC 31556). BRAZIL, Paraná, Guarapuava, road to Turvo, on living leaves of *B. subalternans*, 12 October 2010, E. Guatimosim (VIC 31573). BRAZIL, Santa Catarina, Morro da Fumaça, road to Urussanga, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31584). BRAZIL, Goiás, Uberaba, road to Nova Ponte, on living leaves of *B. subalternans*, 06 December 2010, L. L. Duarte (VIC 31598). BRAZIL, Goiás, Pantaninho, on living leaves of *B. subalternans*, 06 December 2010, L. L. Duarte (VIC 31600).

Entyloma compositarum Farlow Bot. Gaz. 8: 275 (1883)

Fig. 22

Lesions on living leaves starting as minute chlorotic dots, becoming circular and flat, 5.0–35 mm, dark center and yellow greenish halo adaxially, greenish center and yellow-greenish halo, abaxially, dry and necrotic with age leading to leaf blight, and death. *Internal mycelium* not seen. *Sori* of loose spores embedded in host tissue. *Ustilospores* globose, isolated, $10\text{--}15\text{--}(18) \times 10\text{--}14 \mu\text{m}$, subhyaline, wall mostly one-layered, regular, *ca* 1.0 μm . *Ustilospore germination* observed

occurring inside the leaf, intercellularly between the host cells. *Anamorph* hypophyllous, forming caespituli through stomata.

Material examined: BRAZIL, Goiás, Anápolis, on living leaves of *B. pilosa*, 03 December 2010, L. L. Duarte (VIC 31553). BRAZIL, Goiás, Monte Carmelo, on living leaves of *B. pilosa*, 06 December 2010, L. L. Duarte (VIC 31561). BRAZIL, Minas Gerais, Patrocínio, road to Ibiá, on living leaves of *B. pilosa*, 07 December 2010, L. L. Duarte (VIC 31550).

Entyloma guaraniticum Speg., Fungi guranitici. Anal. Soc. Cient. Argent. 17(3): 128 (1884)

Figs. 23, 24, 25

Lesions on living leaves, starting as minute chlorotic dots, becoming circular convex yellow blisters of hypertrophied tissue, 1.0–6.0 mm diam, yellowish becoming dark brown, dry and necrotic with age either leading to perforation of leaf or coalescing and leading to leaf blight, and death. *Sori* densely packed and embedded in host tissue. *Internal mycelium* not seen. *Ustilospores* sphaerical, subglobose to ellipsoid, often becoming distorted, subangular and irregular, (13–)15–18(–20) × (10–)12–15 µm, subhyaline to yellowish-brown, wall two-layered, the inner layer regular and thin, *ca* 1.0 µm, the outer, strongly irregular, (1.0)–2.0–3.0(–4.0) µm thick, smooth. *Anamorph* hypophyllous, forming caespituli through stomata, sickle-shaped and needle-shaped sporidia (10–)13–20(–24) × 2.0–3.0 µm, strongly guttulate, hyaline, smooth, needle-shaped sporidia 45–50 × 1.0–2.0 µm, guttulate, hyaline, smooth. *Germination* of the *Tilletia* type, with immediate conjugation of the sessile basidiospores, and development of needle-shaped sporidia.

Material examined: BRAZIL, Minas Gerais, Viçosa, UFV (Clínica de Doenças de Plantas), on living leaves of *B. pilosa*, 15 August 2009, E. Guatimosim (VIC 31504). BRAZIL, Minas Gerais, Viçosa, UFV (Gado de Leite), on living leaves and stems of *B. pilosa*, 15 August 2009, E. Guatimosim (VIC 31506). BRAZIL, Minas Gerais, Patos de Minas, BR-365 highway, on living leaves of *B. pilosa*, 11 February 2010, E. Guatimosim (VIC 31535). BRAZIL, Goiás, São Simão, on

living leaves of *B. subalternans*, 12 February 2010, B. S. Vieira (VIC 31565). BRAZIL, São Paulo, Tietê, (IAC), on living leaves of *B. pilosa*, 23 April 2010, E. Guatimosim (VIC 31520). BRAZIL, Paraná, Mamborê, road to Campo Mourão, on living leaves of *B. subalternans*, 13 October 2010, E. Guatimosim (VIC 31571) BRAZIL, Rio Grande do Sul, Taquara, road to Santo Antunes, on living leaves of *B. pilosa*, 16 October 2010, E. Guatimosim (VIC 31526). BRAZIL, Santa Catarina, Morro da Fumaça, road to Coal do Sul, on living leaves of *B. subalternans*, 17 October 2010, E. Guatimosim (VIC 31583). BRAZIL, Minas Gerais, Unaí, road to Cristalina, on living leaves of *B. subalternans*, 28 November 2010, L. L. Duarte (VIC 31595). BRAZIL, Goiás, Guaianira, on living leaves of *B. pilosa*, 04 December 2010, L. L. Duarte (VIC 31554). BRAZIL, Goiás, Inacionlândia, road to Itumbiara, on living leaves of *B. pilosa*, 05 December 2010, L. L. Duarte (VIC 31551). BRAZIL, Goiás, Rio Verde, road to Santa Helena, on living leaves of *B. subalternans*, 05 December 2010, L. L. Duarte (VIC 31593). BRAZIL, Goiás, Goiânia, road to Guaianira, on living leaves of *B. pilosa*, 05 December 2010, L. L. Duarte (VIC 31552). BRAZIL, Minas Gerais, Monte Carmelo, road to Coromandel, on living leaves of *B. pilosa*, 06 December 2010, L. L. Duarte (VIC 31559). BRAZIL, Minas Gerais, Coromandel, on living leaves of *B. pilosa*, 06 December 2010, L. L. Duarte (VIC 31560). BRAZIL, Minas Gerais, Patrocínio, road to Ibiá, on living leaves of *B. pilosa*, 07 December 2010, L. L. Duarte (VIC 31549).

Discussion

Anthracnose

Colletotrichum dematium (Pers.) Grove was the sole species in this genus previously recorded on *Bidens* (on *Bidens pilosa*, in Cuba, Venezuela, and West Indies) [17, Tab. 1]. The fungus found causing anthracnose on *B. subalternans* is clearly different from *C. dematium*. It has straight to slightly curved conidia with rounded apices, and shorter and wider conidia (7–)9–18(–21) × (3–)4–5(–6) µm in the material collected in Brazil and 19.5–24 × 2–2.5(–3.5) µm for *C. dematium* [18]. There are ongoing cultural and molecular studies aimed at clarifying the identity of this fungus. Morphology-based taxonomy of *Colletotrichum* has been

proved in recent years to be inadequate and unreliable [19, 20]. Taxonomy of *Colletotrichum* is presently at a stage of transition and it is therefore regarded as inadequate the proposition of a new taxon to accommodate the anthracnose fungus on *Bidens* here. The results of the pathogenicity studies obtained by the group based at the Universidade de Patos de Minas indicated that this fungus has high biocontrol potential and is capable of quickly killing the plants in inundative inoculum applications. At 7 days after inoculation, inoculated plants were dry and smaller than controls. Stems were deformed, the leaves were necrotic, and the fungus also affected the production of seeds. Eighth days after inoculation, all the plants were dead. The limited list of mycoherbicides that were already registered and/or reached the market worldwide (of only 12 products) included four which are based on species of *Colletotrichum* [13], further supporting the potential of the newly found isolate of *Colletotrichum* as a biocontrol agent of *Bidens*.

Downy mildew

Two species of *Plasmopara* have been described occurring on *Bidens*, *Plasmopara angustiterminalis* f. *bidentis* Novot. and the cosmopolitan *Plasmopara halstedii* (Farl.) Berl. & De Toni.

The taxonomy of *P. halstedii* is very difficult since the pathogen can cause downy mildew infection on more than 80 genera of the subfamilies Asteroideae and Cichorioideae of the Asteraceae [21]. Attempts have been made to split up the *P. halstedii* complex into various new taxa linked to supposed host-specificity (for summary see [21]). However, cross infection studies for the determination of host specificity were mostly lacking and this splitting of the species was questioned by later authors. Nowadays, the taxonomic classification is based on physiological, morphological, chemical and molecular phylogenetic characters. One such based study [22] had separated *Plasmopara angustiterminalis*, the downy mildew pathogen on *Xanthium strumarium* (common cocklebur) from *Plasmopara halstedii*. A later study [23], on the same taxon, found morphological differences between *P. angustiterminalis* and *P. halstedii*, supporting the distinction of the new species. Nevertheless another study [24] regarded the isolate from *X. strumarium* as conspecific with *P. halstedii* due to morphological similarity and

results of cross infection studies. The situation is further confused by a more recent work [25], based on ITS-2 sequences, showing that the separation of sunflower downy mildew pathogens into specialized subgroups pathogenic to perennial or annual hosts as suggested by [22] is not correct, disagreeing with [23], who shows that ITS-2 clearly separates these two species. All considered, it was then decided that a more conservative approach should be taken for the moment with regards to the downy mildews of *Bidens* and to identify these as *P. halstedii* sensu lato.

Plasmopara halstedii had already been described on several species of *Bidens*, namely *B. cernus*, *B. comosa*, *B. connate*, *B. frondosa*, *B. laevis*, *B. pilosa*, and *B. vulgata* [26, 27]. On *B. pilosa* it had been recorded only in the United States [27] but it has never been recorded before on *B. subalternans*. This fungus is an important pathogen of sunflower (*Helianthus annuus* L), in several countries, and also affects more than 80 genera of the family Asteraceae, which has many commercial flowers. Unless host-specificity is clearly demonstrated for strains attacking plants in the *B. pilosa* complex this species cannot be considered further for use in biological control. Its use, in case such host-specific strains are found, would be limited to use in classical biological control, as this is an unculturable biotroph with little or no potential for mycoherbicide development. Of relevance for classical biocontrol, would be the fact that strains of this fungus attacking *Bidens* appears to be presently restricted to the Americas.

Leaf spots

Three species of *Cercospora* are listed as occurring on the genus *Bidens*: *Cercospora bidentis* Tharp.; *Cercospora bidentis-biternatae* Pavgi & U.P. Singh; *Cercospora maculicola* Thirum. & Govindu. There is also one record of *Cercospora coffeicola* Berk. & Cooke (the eye-spot pathogen of coffee) on *Bidens*, but it is to be regarded, nevertheless, as doubtful and requiring confirmation. It is based on a single obscure publication and would have involved an unusual and abnormal host-jump from an unrelated host (coffee) to *B. pilosa*. Nevertheless, this fungal species is not kept in the list of *Cercospora* species on the *B. pilosa*-complex in the present discussion. The others three species have

clear morphological differences separating them. None of these fungal species has ever been reported on *B. subalternans*. Seventeen samples of *Bidens subalternans* bearing *Cercospora* colonies were collected throughout the year at all regions covered during this survey making this the most common group of fungal pathogens collected on this host. They were identified as belonging to two species, *C. bidentis* and *C. maculicola*. Their separation based only in terms of field symptoms is difficult, but based on sporulation and morphology is very easy.

Morphology of *C. bidentis*, *C. bidentis-biternatae*, and *C. maculicola* is clearly distinct. Conidiophores of *C. bidentis* and *C. bidentis-biternatae* are bigger than those of *C. maculicola*, ($36\text{--}70 \times 3.5\text{--}6.0 \mu\text{m}$, 2–4 septate in *C. bidentis*, $9.0\text{--}33 \times 2.5\text{--}4.5 \mu\text{m}$, nongeniculate in *C. bidentis-biternatae* and $24\text{--}64 \times 4.0\text{--}6.5 \mu\text{m}$, rarely 1-septate in *C. maculicola*). The sub-geniculation at the tip, in *C. maculicola* (absent in the others two) is an easily recognizable useful feature for distinction. Finally, the shape and septation of the conidia is also distinct for these species: obclavate to acicular, slightly sinuous, 2–20 septate in *C. bidentis*, clavate, straight to slightly bent, 0–5 septate in *C. bidentis-biternatae*, and obclavate to cylindrical, once-curved, 1–10 septate in *C. maculicola*.

Cercospora bidentis had already been described on the following species of *Bidens*: *B. bipinnata*, *B. biternata*, *B. cernua*, *B. coronata*, *B. laevis*, *B. nashii*, and *B. pilosa* [27, 28, 29; 30, 31]. On *B. pilosa*, it has been recorded in many countries, including Brazil [32]. *Cercospora maculicola* is known only from *Bidens pilosa* in India [33]. This is the first record of *C. maculicola* on *B. pilosa* in Brazil, and it is both the first record of *C. bidentis* and of *C. maculicola* on *B. subalternans*.

These two *Cercospora* species were pathogenic to both *Bidens* species, but the symptoms were observed only in older leaves. No symptoms were observed on stems or new leaves and it appears that seed production was unaffected by attack from these fungi, as suggested by observations in the field. Additional research of impact of these two species on their hosts under controlled conditions are required for further evaluating their biocontrol potential.

Twelve species of *Pseudocercospora* have been recorded on members of the family *Asteraceae*. None of these records was on a member of the genus *Bidens*. They are: *Pseudocercospora achilleae-millefolii* U. Braun; *Pseudocercospora angustana* (Ferraris) U. Braun; *Pseudocercospora eleonora-reginae* (Bubák & Malkoff ex Höhn.) U. Braun; *Pseudocercospora eupatorii* Bhalla, Arch. Singh & S.K. Singh; *Pseudocercospora harcynica* U. Braun; *Pseudocercospora helianthellae* (Ellis & Everh.) U. Braun; *Pseudocercospora hieracii* (Jaap) U. Braun; *Pseudocercospora koelpiniae* (Annal.) U. Braun; *Pseudocercospora latifoliae* U. Braun & Crous; *Pseudocercospora nivea* (Ellis & Barthol.) Deighton; *Pseudocercospora septorioides* (Sacc.) U. Braun; *Pseudocercospora tragopogi* U. Braun. [34, 35].

Pseudocercospora **sp. nov.** is the first fungus belonging to this genus to be recorded on a member of the genus *Bidens* and differs from all other species of *Pseudocercospora* on *Asteraceae* in having well developed stromata, slightly pigmented, amphigenous caespituli of conidiophores arising through the stomata and hence is proposed as a new species for the genus.

None of the attempts to promote *in vitro* sporulation of this fungus was successful, neither were the attempted inoculations with mycelial suspensions. Further studies as in addition to [36] are necessary to clarify the potential of this fungus as a biocontrol agent.

Powdery mildews

Podosphaera fusca (Fr.) U. Braun & Shishkoff. The anamorph of *P. fusca* is *Oidium citrulli* Yen & Wang, and it is very similar to the anamorph of the other powdery mildew on *B. pilosa* and *B. subalternans* – *Oidium asteris-punicei* Peck (teleomorph: *G. cichoracearum* var. *cichoracearum*) Their separation based only in terms of field symptoms is impossible. The main distinguishing morphological difference between these two species' anamorphs is on conidial germination: germ tubes arising from an end, moderately long, simple, with enlarged but unlobed tips - in *G. cichoracearum* var. *cichoracearum*, and arising from the middle of the conidium, short, bifurcate, and having a lobed tip in *P. fusca*. The

presence of fibrosin bodies can be an useful distinguishing feature for species delimitation in this fungus group but no information was provided for this feature for *G. cichoracearum* var. *cichoracearum* in the main monograph [37].

Golovinomyces cichoracearum var. *cichoracearum* is only known from *B. subalternans* in Argentina [38]. This is the first record of *G. cichoracearum* var. *cichoracearum* on *B. pilosa*, and the first record on *B. subalternans* in Brazil. It occurred mainly during the summer in southeastern and central Brazil. *Podosphaera fusca* is a polyphagous species of powdery mildew attacking numerous genera of the Asteraceae and having been recorded on several species of *Bidens*: *B. bipinnata*, *B. biternata*, *B. cernua*, *B. dahlioides*, *B. formosa*, *B. frondosa*, *B. palmivora*, *B. pilosa*, *B. tripartita*, *B. vulgate* [39, 40, 41, 42]. On *B. pilosa*, it had already been recorded in China and India [42, 43]. This is the first record of this fungus on *B. pilosa* in Brazil, and the first record on *B. subalternans*, occurring mainly during the summer in southeast of the country. The combination of their broad host-range, the biotrophic nature of the powdery mildews and the relatively mild impact on their hosts, as observed in the field, excludes this group of fungi for further considerations for use in biological control of *B. pilosa* and *B. subalternans*.

Rusts

Six species of Pucciniales are known in association with members of the genus *Bidens*, four of which belonging to the genus *Uromyces*: *Uromyces bidenticulata* (Henn.) Arthur; *Uromyces bidenticola* Arthur; *Uromyces bidentis* Lagerh. and *Uromyces martini* Farl. *U. bidentis* and *U. bidenticola* are the only species of *Uromyces* on *Bidens* occurring in the Western Hemisphere [44]. *Uromyces bidenticola* (anamorph *Uredo bidentis* Henn.) is a macrocyclic, autoecious species widespread in tropical and subtropical regions of the world where its weedy hosts (particularly *Bidens pilosa*) occur. *Uromyces bidentis*, on the other hand, is a microcyclic rust having many morphological features in common with *U. bidenticola* [45]. It also has a broad world distribution, as indicated by records from the American continent but also from Africa, Europe, Asia and Australasia. Their separation in field situations, based on symptom observation is easy: *U.*

bidenticola producing numerous aggregated pustules and *U. bidentis* producing few well spaced pustules on leaves. It is possible that the two species are in fact conspecific and that the differences in life cycle are environment controlled. Further work including inoculation under controlled conditions and molecular phylogenetic tools may clarify the status of these two rust species, but these are outside the scope of the present work. Both species have already been recorded on *B. pilosa* in Brazil [44].

Uromyces bidentis had already been described on the following species of *Bidens*: *B. andicola*, *B. cynapiifolia*, *B. expansa*, *B. leucantha*, and *B. pilosa* [44, 45, 46, 47]. Here, the first record of *U. bidentis* on *B. subalternans* is provided.

The lack of obvious impact of the rust fungi on the two species of *Bidens* and the biotrophic nature of rusts discourages them being considered further for use as biocontrol agents, particularly as mycoherbicides. Their present world wide distribuion also may indicate that their use as classical biocontrol agents would not be effective. It may, nevertheless, be premature to exclude these species from the list of potential classical biocontrol agents as there may exist more damaging strains which might be useful for introduction or a hidden effect of rust infections (such as loss of vigour and competitiveness or reduced seed production in infected plants) which could not be perceived in brief field observations of the disease. Studies involving the use of high inoculum load and comparison of different strains would be useful for clarifying this point. There are many examples of successful use of rusts in classical biological control, such as of *Maravalia cryptostegia* (Cummins) Ono against *Cryptostegia grandiflora* Roxb. Ex R. Br. [11] and *Puccinia chondrillina* Bubak & Sidenham against *Chondrilla juncea* L. [48] and these fungi are often regarded as first choice when a classical biocontrol program of a weed involving pathogens is initiated.

Scab

Sphaceloma bidentis is a poorly known fungal species which was originally described as the cause of scab disease on *Bidens pilosa*. The name anthracnose maculada do picão was used for this disease in the original and sole publication

dealing with this fungus [49]. The description provided by this author, contains detailed information on disease symptoms but little information on fungal morphology and no information on cultural aspects. An emended description containing missing information on this taxon is, therefore, provided as well as more detailed illustrations of this fungus. This is the first record of *S. bidentis* on *B. subalternans*.

This fungus appears to have great potential for use in biocontrol. Inoculations involving the use of conidia were ineffective but those involving the use of mycelial suspension led to severe diseases on the leaves. The leaf spots started as minute depressed dots, becoming vein-delimited, coalescing and expanding towards the leaf edges, leading to entire leaf blight. The fungus was pathogenic to both *Bidens* species. To better understand the real potential of this species, further work involving tests of different culture media and methodologies for mass production, as well as different methods of inoculation are necessary.

White smuts

Five species of *Entyloma* are known to cause white smut on *Bidens*: *Entyloma bidentis* Henn.; *Entyloma compositarum* Farl.; *Entyloma frondosa* Vánky; *Entyloma guaraniticum* Speg.; and *Entyloma spegazzinii* Speg. According to the more recently published information [50], only four species are now known to cause white smut on *Bidens*, since specimens of *E. compositarum* occurring on *B. frondosa*, *B. laevis*, *B. tenuisecta* and *B. vulgata*, in the United States were recognized as *E. frondosa*. But the one-layered wall of ustilospores, which are loosely formed between the host cells, is typical for *E. compositarum* as described in another publication [51], and it is different from the description in the published information [50] of *E. frondosa*. However, the sori appearance (convex, blister or cup-like shaped in *E. guaraniticum* versus flat circular pale leaf-spots on the other species) is sufficient for an easy recognition of *E. guaraniticum* [50]. The taxonomic delimitation for the others species is based almost completely on ustilospore size, which is clearly inadequate once there is infra-specific variation and biometric overlapping for this key feature. Further work, based on molecular phylogenetic tools is clearly necessary to clarify the status of *Entyloma* on *Bidens*.

This would benefit from the fact that *Entyloma* grows in culture, readily producing yeast-like colonies from which, DNA is likely to be easily extracted. The main differentiating characters of the *Entyloma* species of *Bidens* are shown (Key 1).

Key 1 – Key to *Entyloma* species of *Bidens*

- 1 Sori convex, blister or cup-like shaped.....*E. guaraniticum*
- 1' Sori flat, circular pale leaf-spots2
- 2 Ustilospores loosely formed between the host cells, one-layered wall.....*E. compositarum*
- 2' Ustilospores densely formed between the host cells, two-layered wall3
- 3 Spores 12–20 µm long, wall 2.0–5.5 µm thick.....*E. spegazzinii*
- 3' Ustilospores smaller, wall thinner.....4
- 4 Ustilospores 11–18(–20) µm, wall 1.0–3.0(–4.0) µm thick.....*E. frondosa*
- 4' Ustilospores 10–16 µm, wall 1.0–1.5(–2.5) µm thick.....*E. bidentis*

Entyloma bidentis had already been recorded on the following species of *Bidens*: *B. bipinnata*, *B. formosa*, and *B. pilosa* [51, 52, 53]. *Entyloma compositarum* had already been recorded on *B. pilosa* in the followings countries: Ecuador, Hawaii (USA), USA, and Venezuela [27, 53, 54]. *Entyloma guaraniticum* had already been described on the following species of *Bidens*: *B. bipinnata*, *B. chilensis*, *B. frondosa*, *B. pilosa*, *B. pilosa* var. *radiata* [50, 53], in the following countries Argentina, Australia, China, Costa Rica, Cuba, Dominican Republic, Hong-Kong, India, Jamaica, Madagascar, Malasya, Malawi, Mauritius, Papua New Guinea, Paraguay, Puerto Rico, Kenya, Sri-Lanka, Sudan, Tanzania, Taiwan, Trinidad & Tobago, Uganda, USA, Virgin Islands, West Indies, Zambia, Zimbabwe (Tab. 1). These are the first records of *E. bidentis* and *E. guaraniticum* causing white smut

on *B. subalternans*, and the first records of *E. compositarum* and *E. guaraniticum* on *B. pilosa* in Brazil.

None of the attempts to demonstrate the pathogenicity of the two *Entyloma* species was successful regardless of the kind of inoculum or method of inoculation utilized. It is known from previous studies involving another fungus in this genus (*Entyloma ageratinae* R.W. Barreto & H.C. Evans) as a weed biocontrol agent that manipulation of white smuts is difficult and the yeast-like sporidial cultures appear to be non-infective [55]. This limitation did not prevent its highly successful use as a classical biocontrol agent in Hawaii [56] and later in New Zealand [57] but discourages further investigations towards mycoherbicide development, unless a breakthrough is achieved on the mass production of infective propagules in vitro.

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Tables

Tab. 1 – List of the species already recorded on *Bidens* complex

Fungi	Distribution (Country)	Host
Oomycota		
Peronosporales		
<i>Albugo</i> sp.	Venezuela	<i>B. pilosa</i>
<i>Phytophthora nicotianae</i> var. <i>parasitica</i>	Hawaii (USA), USA	<i>B. pilosa</i>
<i>Plasmopara halstedii</i>	USA	<i>B. pilosa</i>
<i>Tetraploa aristata</i>	Cuba, West Indies	<i>B. pilosa</i>
Ascomycota		
Botryosphaeriales		
<i>Lasiodiplodia theobromae</i>	Cuba, West Indies	<i>B. pilosa</i>
<i>Phyllosticta</i> sp.	Brazil, Cuba, Venezuela, West Indies	<i>B. pilosa</i>
Diaporthales		
<i>Diaporthe phaseolorum</i> var. <i>meridionalis</i>	Brazil	<i>B. pilosa</i>
Erysiphales		
<i>Erysiphe lamprocarpa</i>	South America	<i>B. pilosa</i>
<i>Golovinomyces cichoracearum</i> var. <i>cichoracearum</i>	Argentina	<i>B. subalternans</i>
<i>Neoerysiphe cumminsiana</i>	Argentina, Brazil	<i>B. pilosa</i> e <i>B. subalternans</i>
<i>Neoerysiphe galeopsidis</i>	Cuba	<i>B. pilosa</i>
<i>Oidium erysiphoides</i>	South America	<i>B. pilosa</i>
<i>Oidium</i> sp.	Brazil, China, Cuba, India, Jamaica, Malawi, Sudan, Tanzania, Venezuela, West Indies, Zimbabwe, Zambia (IMI 42267)	<i>B. pilosa</i>
<i>Podosphaera fuliginea</i>	China, India, South Africa	<i>B. pilosa</i>
<i>Podosphaera fusca</i>	China, India	<i>B. pilosa</i>
<i>Podosphaera macularis</i>	USA, Puerto Rico, Virgin Islands, West Indies,	<i>B. pilosa</i>
Capnodiales		
<i>Cercoseptoria</i> sp.	India (IMI 135892)	<i>B. pilosa</i>

<i>Cercospora bidentis</i>	Brazil, cosmopolite (China, Cuba, Ghana, Guinea, India, Kenya, Laos, Malawi, Malaysia, Mauritius, Papua New Guinea, Republic of The Congo, Samoa, South Africa, Taiwan, Thailand, Trinidad & Tobago, USA, Venezuela, West Indies, Zambia)	<i>B. pilosa</i>
<i>Cercospora coffeicola</i>	India	<i>B. pilosa</i>
<i>Cercospora maculicola</i>	India	<i>B. pilosa</i>
<i>Cercospora</i> sp.	India (IMI 135892)	<i>B. pilosa</i>
<i>Isariopsis indica</i> var. <i>cynamopsidis</i>	India	<i>B. pilosa</i>
<i>Mycosphaerella</i> sp.	Cuba, Venezuela, West Indies	<i>B. pilosa</i>
<i>Pseudocercospora megalopotamica</i>	Argentina, China, Colombia, Hawaii (USA), Uganda, USA	<i>B. pilosa</i>
<i>Septocylindrium concomitans</i>	China	<i>B. pilosa</i>
<i>Septoria balansae</i>	Colombia	<i>B. pilosa</i>
<i>Cladosporium oxysporum</i>	West Indies	<i>B. pilosa</i>
<i>Cladosporium</i> sp.	Kenya (IMI 131266)	<i>B. pilosa</i>
Hypocreales		
<i>Fusarium moniliforme</i>	USA	<i>B. pilosa</i>
<i>Mycrothecium leuchotrichum</i>	South Africa (IMI 278126)	<i>B. pilosa</i>
<i>Stachybotrys kampalensis</i>	West Indies	<i>B. pilosa</i>
Incertae sedis - Dothideomycetes		
<i>Schiffnerula pulchra</i>	Cuba, West Indies	<i>B. pilosa</i>
Incertae sedis - Pezizomycotina		
<i>Gyrothrix</i> sp.	West Indies	<i>B. pilosa</i>
Incertae sedis - Sordariomycetes		
<i>Arthrimum</i> sp.	West Indies	<i>B. pilosa</i>
<i>Colletotrichum dematium</i>	Cuba, West Indies, Venezuela	<i>B. pilosa</i>
<i>Colletotrichum gloeosporioides</i> f. sp. <i>pilosae</i>	India	<i>B. pilosa</i>
<i>Colletotrichum</i> sp.	Cuba, USA, Venezuela	<i>B. pilosa</i>
<i>Glomerella cingulata</i>	India, Venezuela (IMI 113592)	<i>B. pilosa</i>
Myriangiales		
<i>Sphaceloma bidentis</i>	Brazil	<i>B. pilosa</i>
<i>Sphaceloma</i> sp.	Zimbabwe	<i>B. pilosa</i>
Pleosporales		
<i>Alternaria cucumerina</i> var. <i>cyamopsidis</i>	South Africa	<i>B. pilosa</i>
<i>Alternaria</i> sp.	Brazil, Venezuela / Zambia (IMI 93901)	<i>B. pilosa</i>

<i>Alternaria zinniae</i>	Kenya, South Africa (IMI 278126)	<i>B. pilosa</i>
<i>Pithomyces bidentis</i>	Taiwan	<i>B. pilosa</i>
<i>Aposphaeria</i> sp.	India (IMI 318751)	<i>B. pilosa</i>
<i>Ascochyta</i> sp.	Australia	<i>B. pilosa</i>
<i>Periconia byssoides</i>	West Indies	<i>B. pilosa</i>
<i>Periconia</i> sp.	West Indies	<i>B. pilosa</i>
Basidiomycota		
Cantharellales		
<i>Rhizoctonia solani</i>	Brazil	<i>B. pilosa</i>
<i>Thanatephorus cucumeris</i>	Brazil	<i>B. pilosa</i>
Entylomatales		
<i>Entyloma bidentis</i>	Brazil, cosmopolite (Australia, Bolivia, Colombia, Costa Rica, India, Kenya, Malawi, Malaysia, Mauritius, Panama, Papua New Guinea, South Africa, Thailand, Taiwan, Tanzania, Zambia, Zimbabwe)	<i>B. pilosa</i>
<i>Entyloma compositarum</i>	Ecuador, Hawaii (USA), USA, Venezuela	<i>B. pilosa</i>
<i>Entyloma guaraniticum</i>	Argentina, cosmopolite (Australia, China, Costa Rica, Cuba, Dominican Republic, Hong-Kong, India, Jamaica, Madagascar, Malaysia, Malawi, Mauritius, Papua New Guinea, Paraguay, Puerto Rico, Kenya, Sri-Lanka, Sudan, Tanzania, Taiwan, Trinidad & Tobago, Uganda, USA, Virgin Islands, West Indies, Zambia, Zimbabwe)	<i>B. pilosa</i>
<i>Entyloma incertum</i>	Ecuador, Venezuela	<i>B. pilosa</i>
<i>Entyloma polysporum</i>	USA, Venezuela, West Indies	<i>B. pilosa</i>
<i>Entyloma spegazzinii</i>	Bolivia, Mauritius, South Africa, Tanzania	<i>B. pilosa</i> & <i>B. subalternans</i>
Sordariales		
<i>Brachysporiella dennisii</i>	Cuba	<i>B. pilosa</i>
<i>Sporidesmiopsis dennisii</i>	West Indies	<i>B. pilosa</i>
Trichosphaeriales		
<i>Nigrospora oryzae</i>	Brazil	<i>B. pilosa</i>
Helotiales		
<i>Botrytis cinerea</i>	China	<i>B. pilosa</i>

<i>Sclerotinia libertiana</i>	Taiwan	<i>B. pilosa</i>
<i>Sclerotinia sclerotiorum</i>	Brazil, China, South Africa	<i>B. pilosa</i>
Pucciniales		
<i>Puccinia obtecta</i>	Cuba, West Indies	<i>B. pilosa</i>
<i>Uredo bidentida</i>	Argentina, Paraguay, Uruguay	<i>B. pilosa</i>
<i>Uredo bidenticola</i>	Brazil, Taiwan	<i>B. pilosa</i>
<i>Uredo bidentis</i>	Australia	<i>B. pilosa</i>
<i>Uredo</i> sp.	Cuba, West Indies	<i>B. pilosa</i>
<i>Uromyces bidenticola</i>	Brazil, cosmopolite (Argentina, Australia, Azores, Bolivia, Brazil, Canary Islands, Cape Verde, Chile, China, Colombia, Costa Rica, Cuba, Dominica, Dominican Republic, Philippines, Grenada, Guatemala, Haiti, Hawaii (USA), Indonesia, Jamaica, Japan, Madagascar, Malaysia, Malawi, Mauritius, Nigeria, New Caledonia, New Zealand, Panama, Papua New Guinea, Puerto Rico, Kenya, Rwanda, Samoa, Sri Lanka, Taiwan, Trinidad & Tobago, Uganda, Uruguay, USA, Venezuela, Vietnam, Virgin Islands, West Indies, Zimbabwe)	<i>B. pilosa</i> e <i>B. subalternans</i>
<i>Uromyces bidenticulata</i>	West Indies	<i>B. pilosa</i>
<i>Uromyces bidentis</i>	Brazil, cosmopolite (Canary Islands, Colombia, Dominican Republic, Ecuador, Philippines, India, Jamaica, New Zealand, Puerto Rico, South Africa, Taiwan, USA, Venezuela, Virgin Islands, West Indies)	<i>B. pilosa</i>
<i>Uromyces densus</i>	Puerto Rico	<i>B. pilosa</i>
<i>Uromyces martinii</i>	USA	<i>B. pilosa</i>
<i>Uromyces</i> sp.	Colombia, West Indies	<i>B. pilosa</i>
Urocystidiales		
<i>Thecaphora denticulata</i>	Mexico	<i>B. pilosa</i>
<i>Thecaphora pustulata</i>	Colombia, Costa Rica, Puerto Rico, Virgin Islands, West Indies	<i>B. pilosa</i>
Incertae sedis		
Incertae sedis		

<i>Sclerotium rolfsii</i>	Zimbabwe	<i>B. pilosa</i>
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Figures

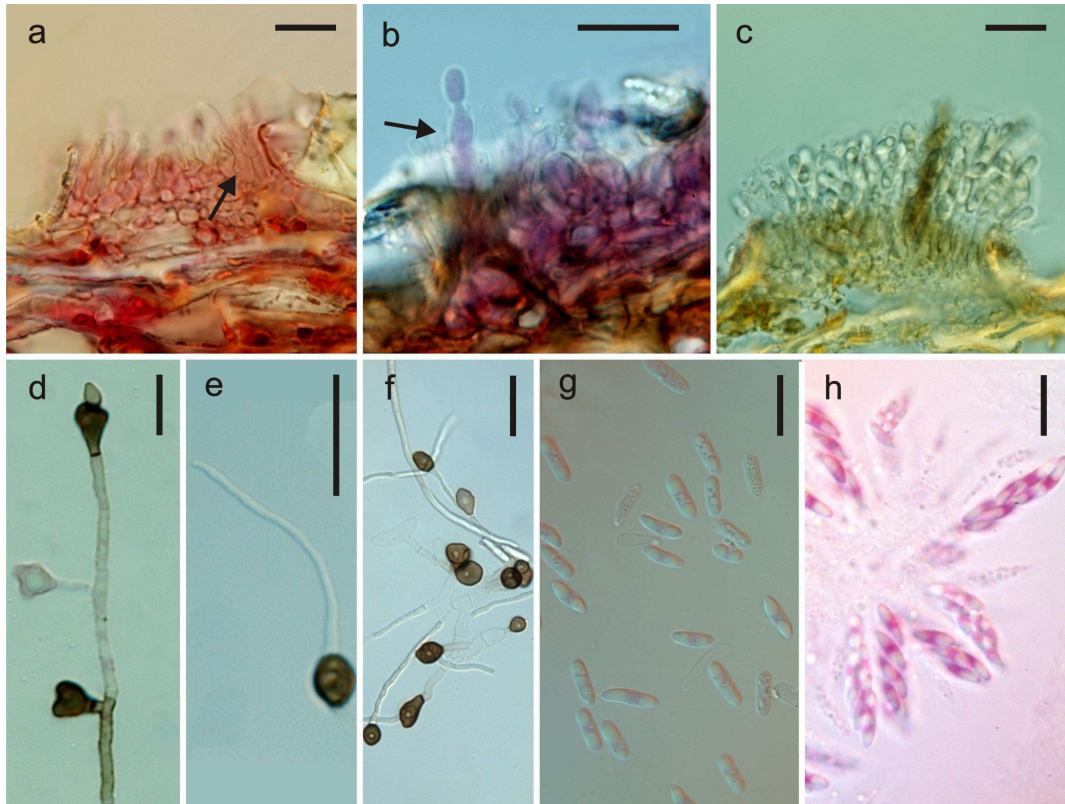


Fig 1 – *Colletotrichum* sp. on *Bidens subalternans*: section through acervuli (note the conidiogenous cell – arrowed) (a-c); appressoria (d-f); conidia (g); asci and ascospores of *Glomerella* sp. produced on PDA (h). Bars = 20.0 μ m.

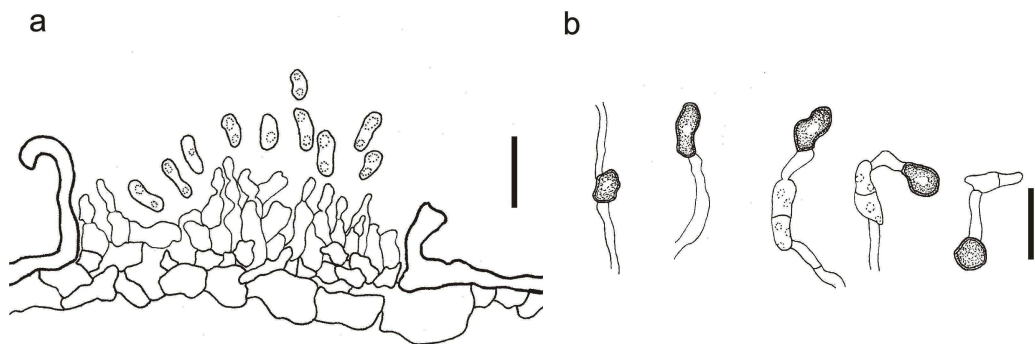


Fig 2 – *Colletotrichum* sp. on *Bidens subalternans*: acervulus (a); appressoria (b). Bars = 20.0 μ m.

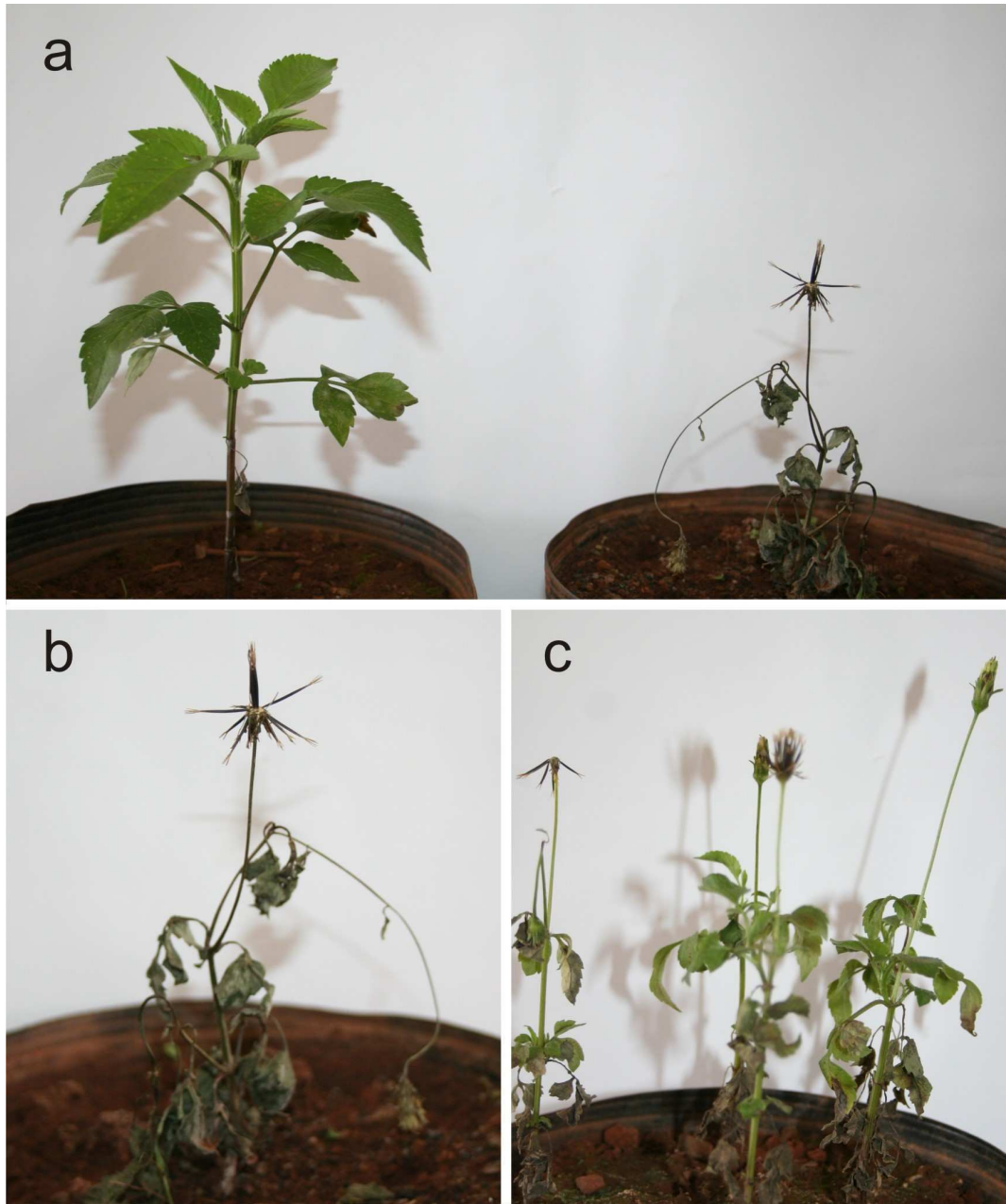


Fig 3 – *Colletotrichum* sp.: Pathogenicity test evaluated at 7 days after inoculation. Treatment and controls (a); deformation on the stems (b); defoliation (c).

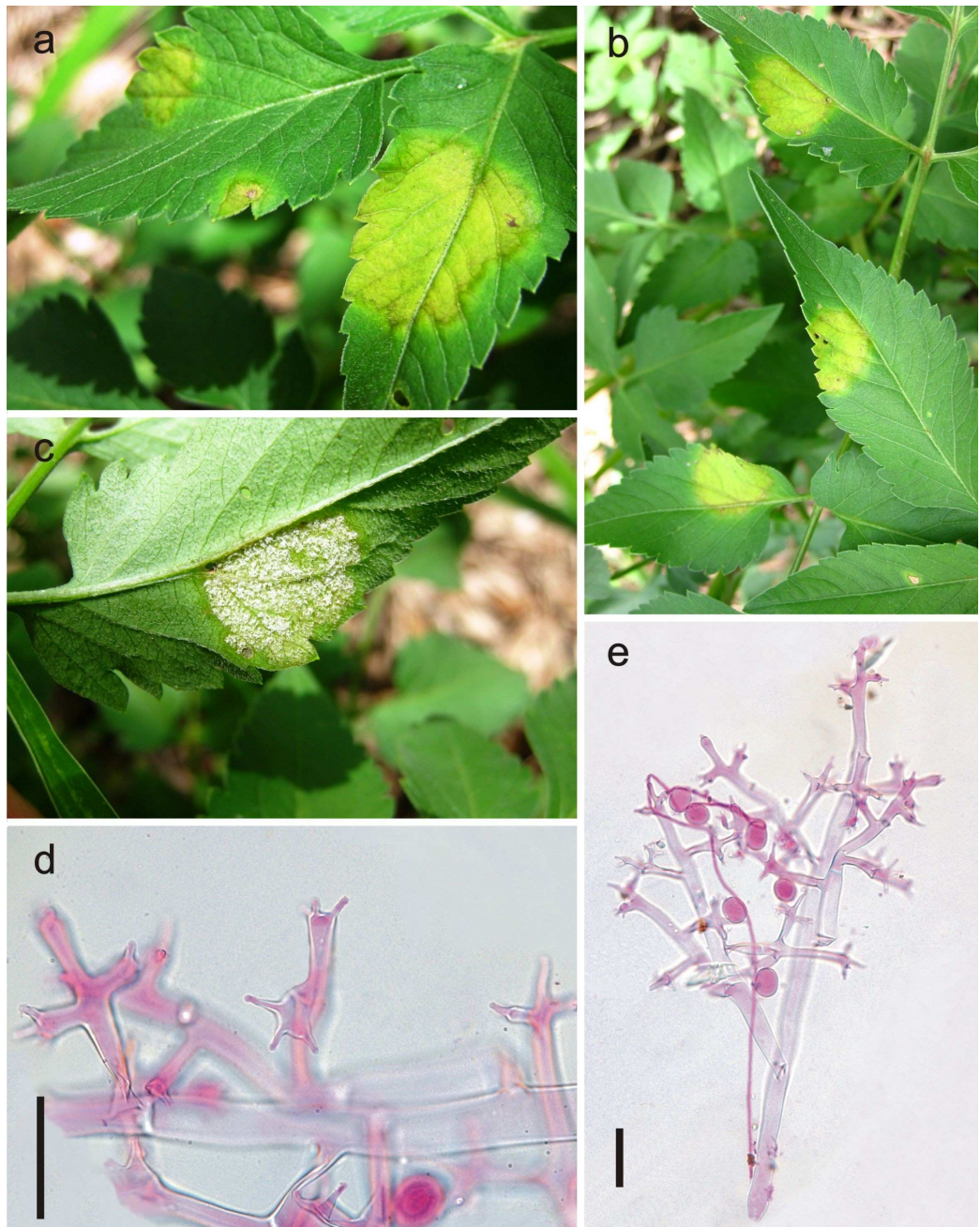


Fig 4 – *Plasmopara halstedii*: blight/downy mildew symptoms (d); trifurcate terminal branches of sporangiophore (d). Bar. = 20.0 μm ; sporangiophore and sporangia (e). Bar. = 40.0 μm .

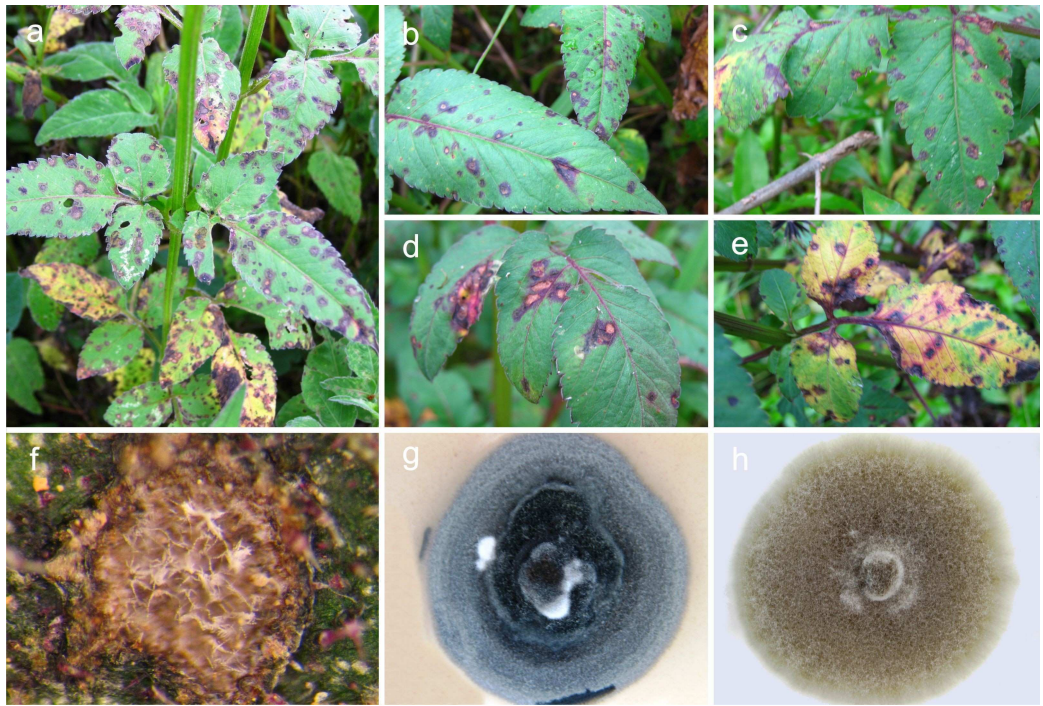


Fig 5 – *Cercospora bidentis*: leaf spots showing the symptoms of the disease (a-e); sporulation on lesion (f); colony on PDA (g); colony on PCA (h).

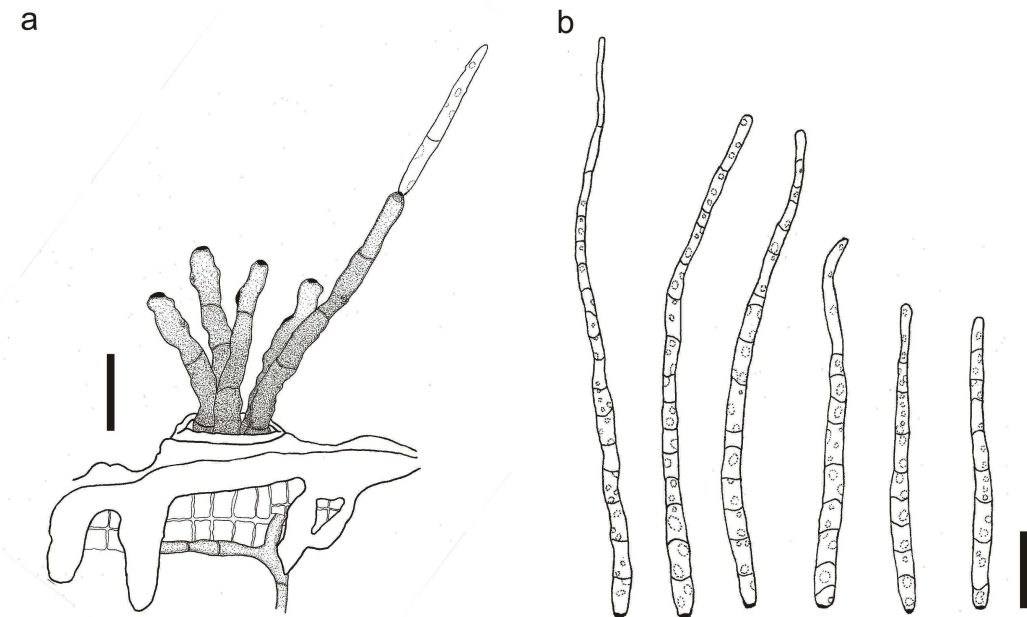


Fig 6 – *Cercospora bidentis*: conidiophores arising through stoma (a); conidia (b). Bars = 20.0 μm

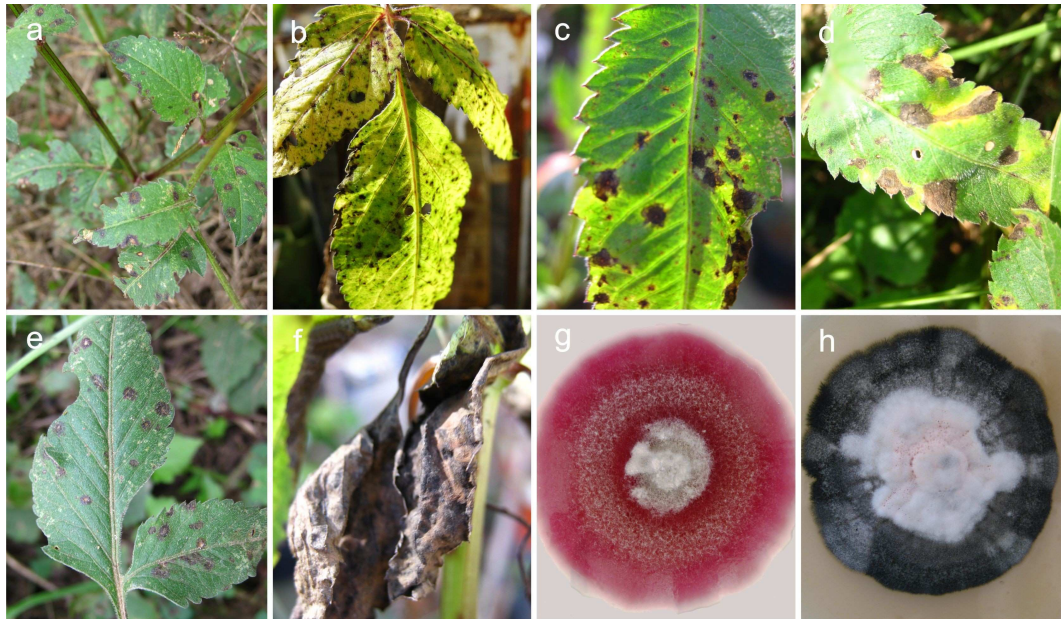


Fig 7 – *Cercospora maculicola*: leaf spot symptoms (a-f); colony on PDA (g); colony on PCA (h).

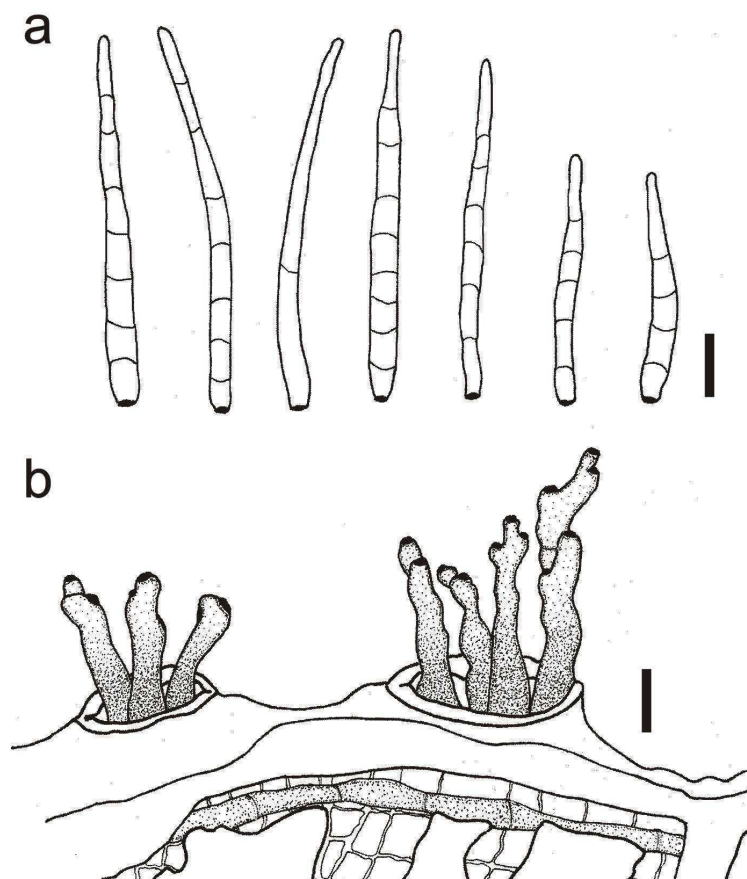


Fig 8 – *Cercospora maculicola*: conidia (a); conidiophores arising through stomata (b). Bars = 20.0 μm .

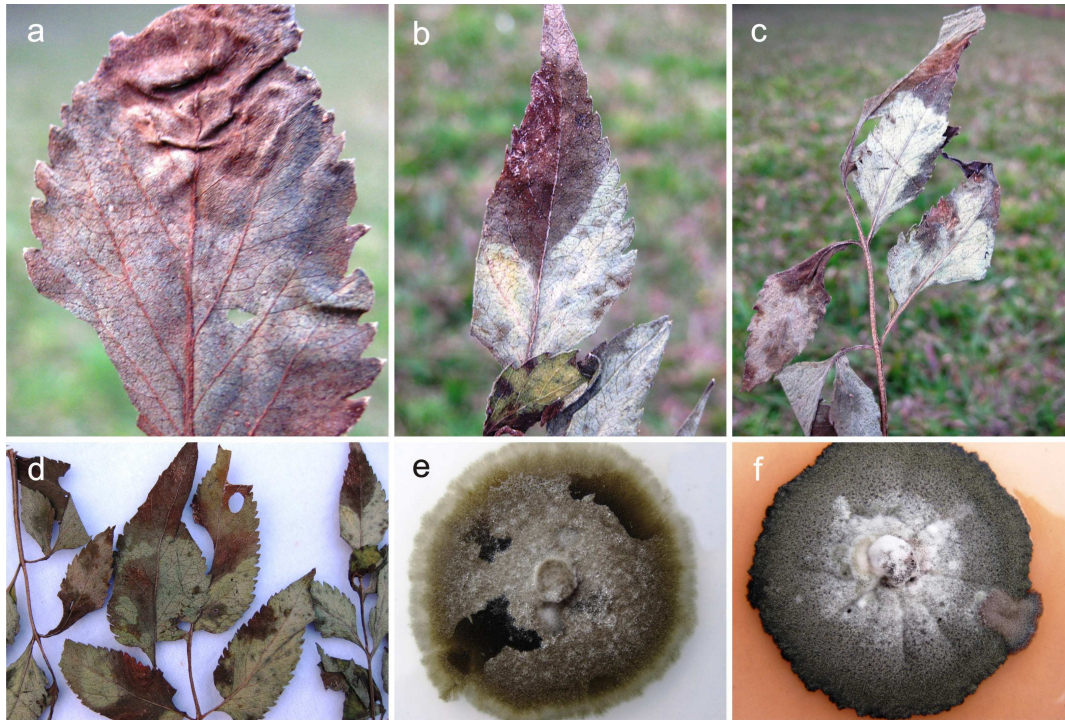


Fig 9 – *Pseudocercospora* sp.nov on *Bidens subalternans*: leaf-spot symptoms (a-d); colony on PDA (e); colony on PCA (f).

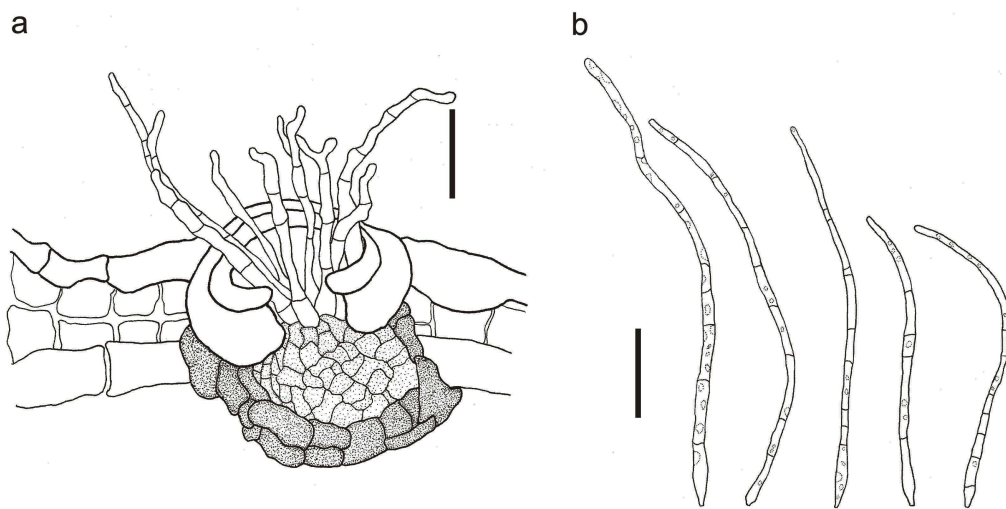


Fig 10 – *Pseudocercospora* sp.nov from *Bidens subalternans*: conidiophores arising from stroma, through stoma (a); conidia (b). Bars = 20.0 μ m.

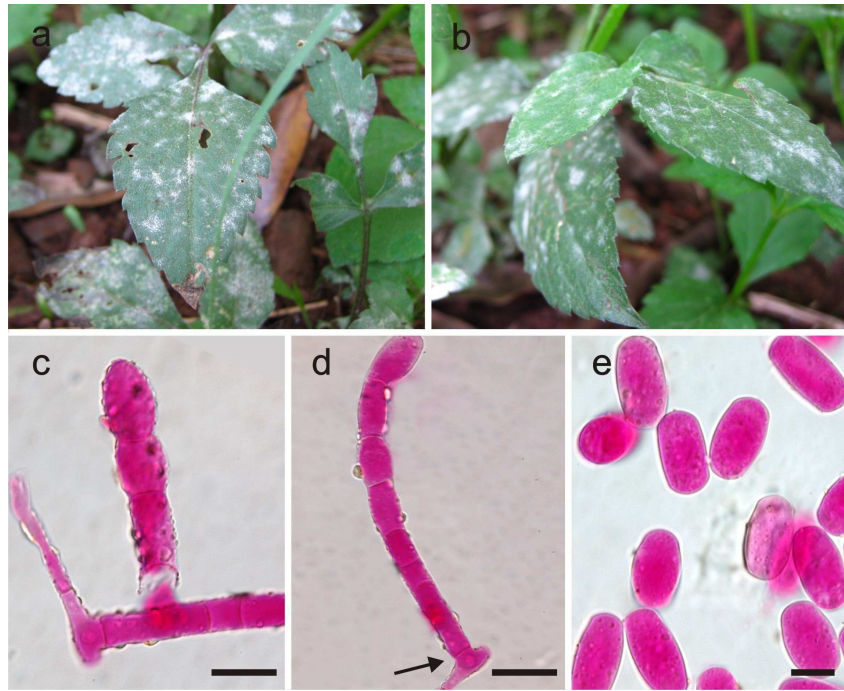


Fig 11 – *Golovinomyces cichoracearum*: powdery mildew symptoms on leaves (a-b); conidiophores (note the constriction at the basal septum (arrowed) (c-d); conidia (e). Bars = 20.0 μ m.

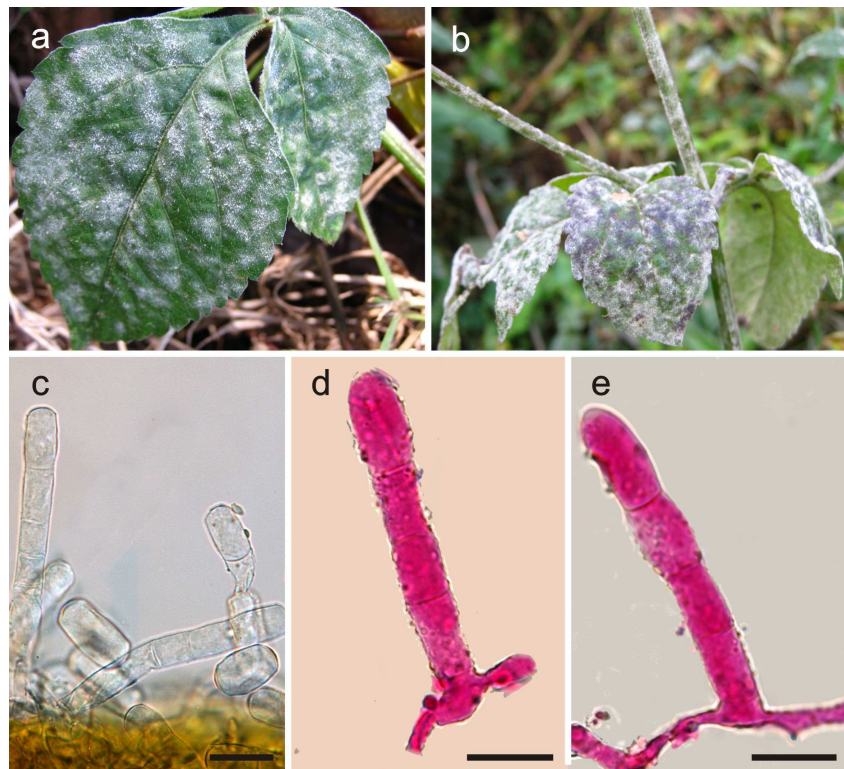


Fig 12 – *Podosphaera fusca*: powdery mildew symptoms on leaves and stems (a-c); conidiophores and conidia (d-e). Bars 20.0 μ m.

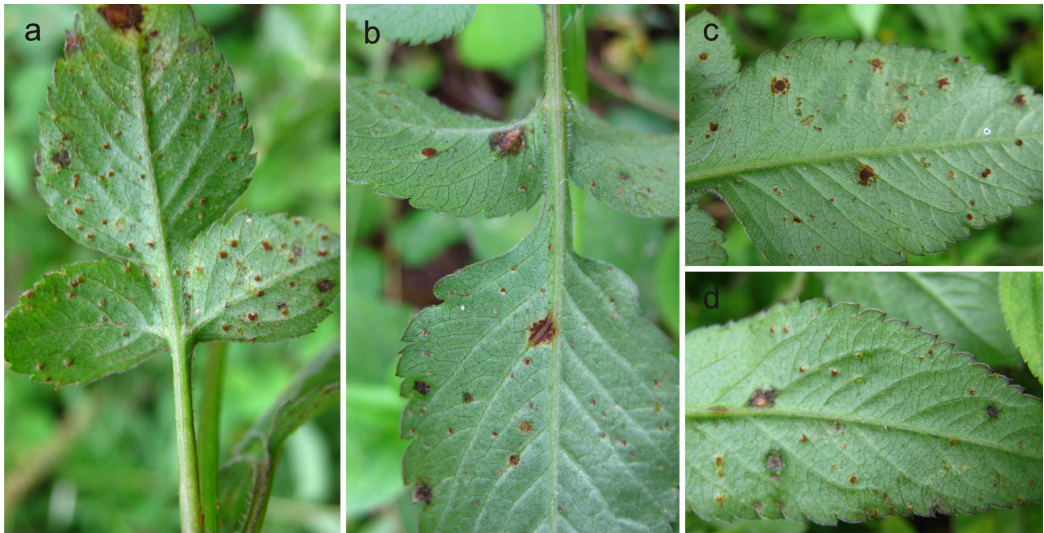


Fig 13 – *Uredo bidentis*: (a-d) rust symptoms on leaves.

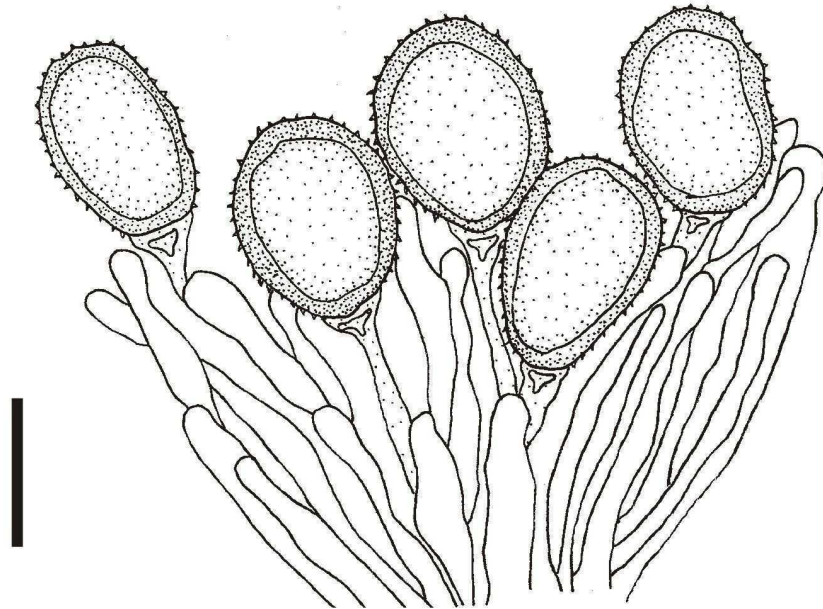


Fig 14 – *Uredo bidentis*: uredinia with urediniospores. Bar = 20.0 μm .

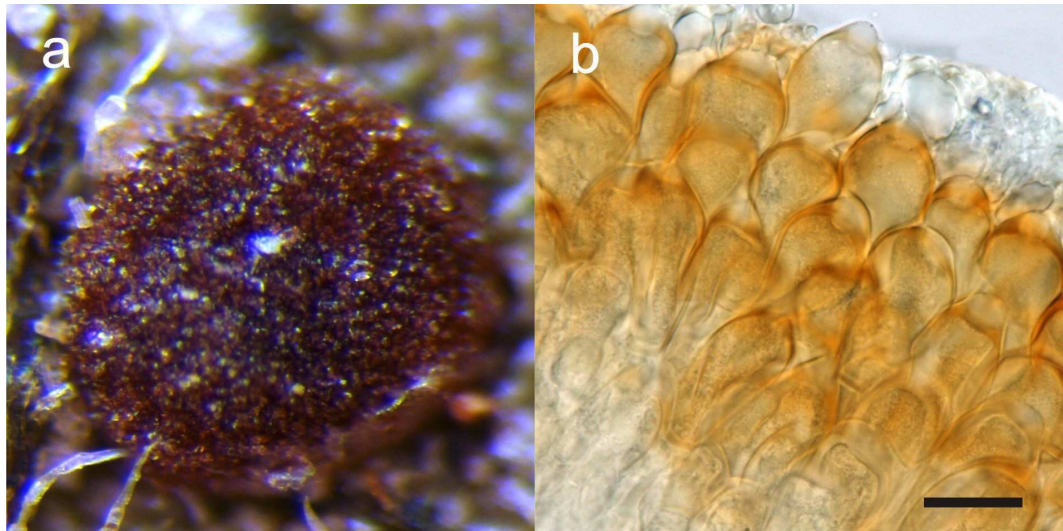


Fig 15 – *Uromyces bidentis*: telia (a); section through telia with teliospores (b).
Bar = 20.0 μm .

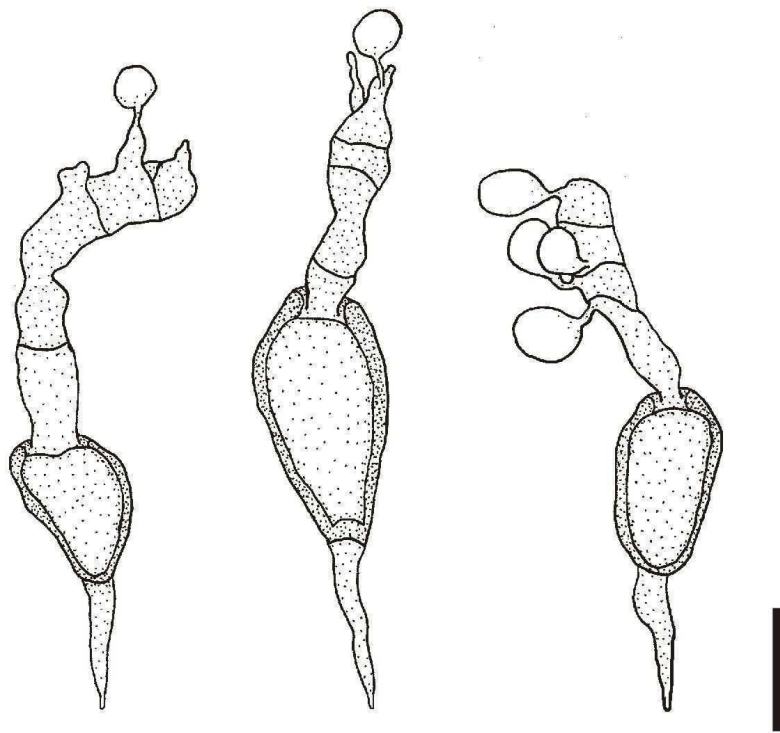


Fig 16 – *Uromyces bidentis*: germination of teliospores. Bar = 20.0 μm .

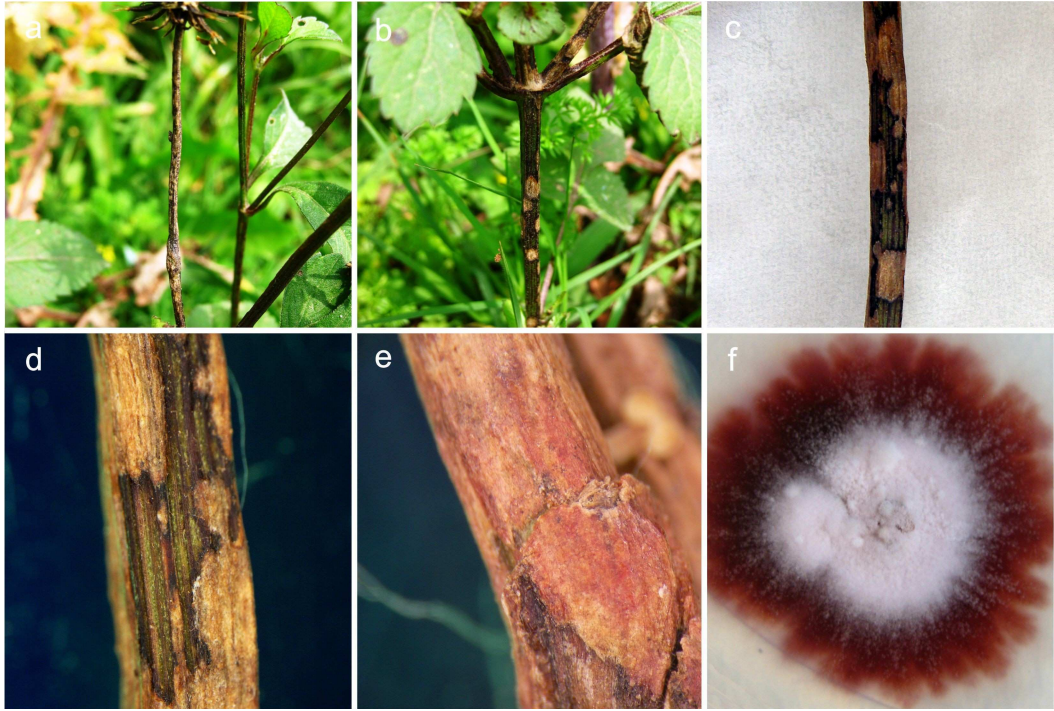


Fig 17 – *Sphaceloma bidentis* on *Bidens subalternans*: scab symptoms on stems (a-e); colony on PCA (f).

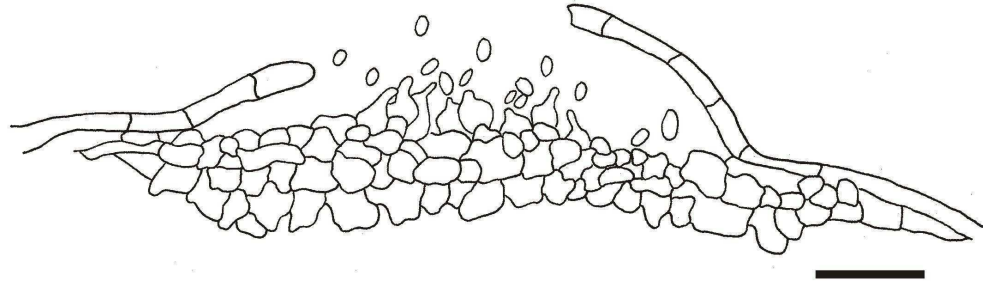


Fig 18 – *Sphaceloma bidentis* on *Bidens subalternans*: section through acervulus showing conidigenous cells and conidia. Bar = 20.0 μm .

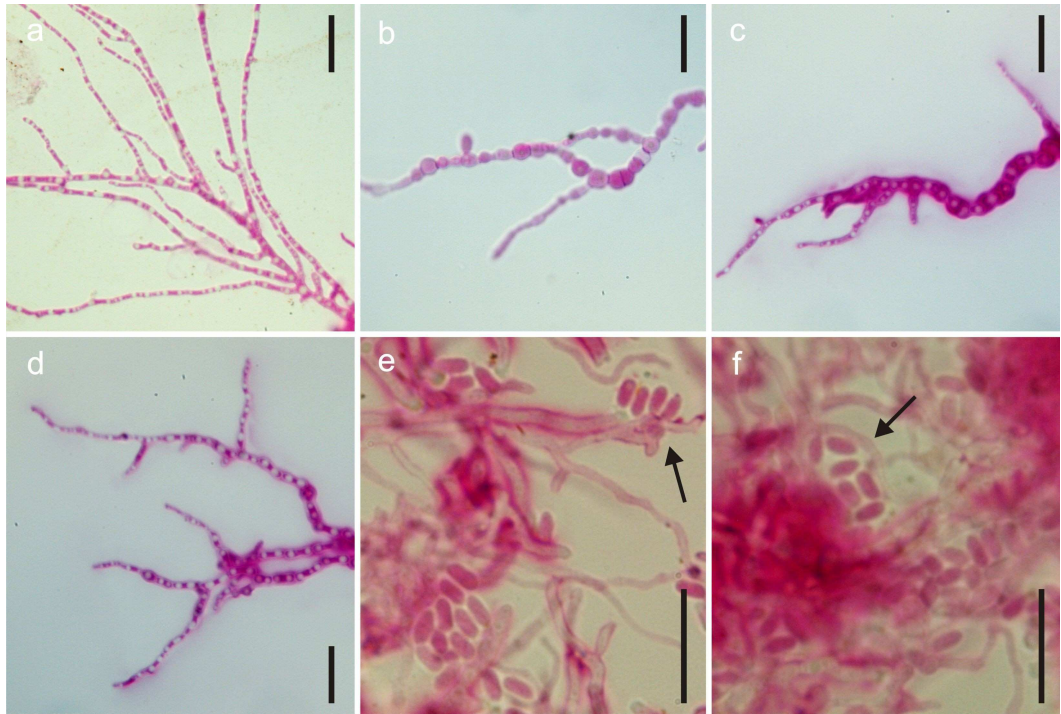


Fig 19 – *Sphaceloma bidentis* on *Bidens subalternans*: mycelium (a); hyphae forming chlamydospores (b-d); conidia on microculture (arrowed) (e-f). Bars = 20.0 μ m.

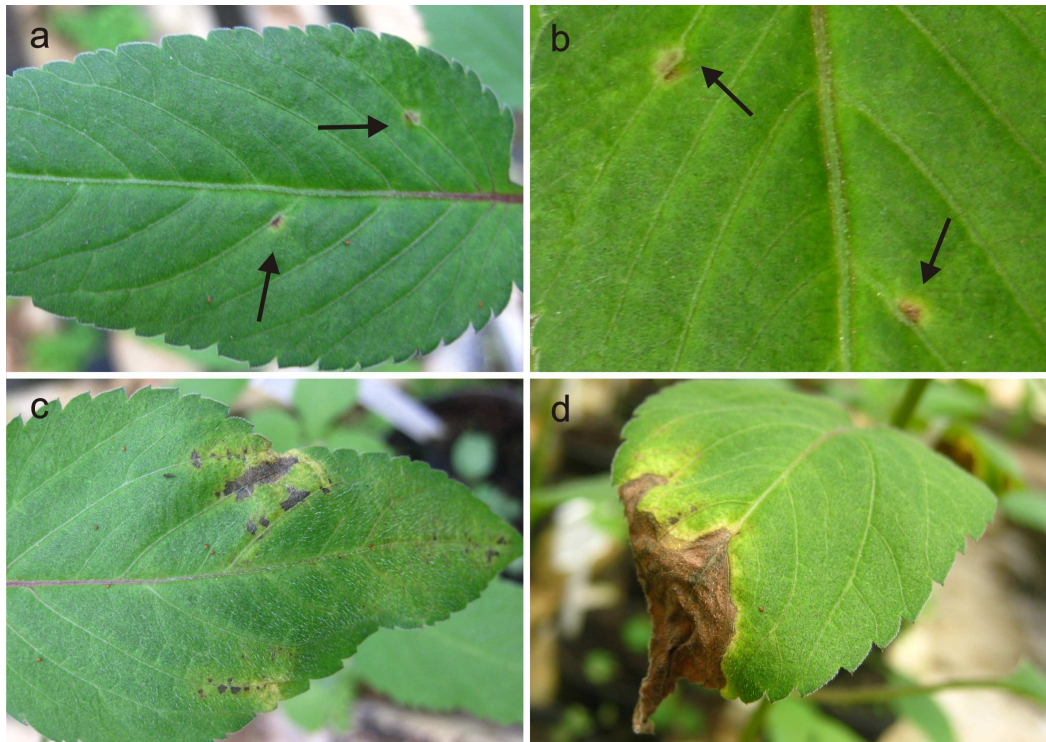


Fig 20 – *Sphaceloma bidentis*: Pathogenicity test evaluated at 22 days after inoculation. Leaf spots progression (a-d).

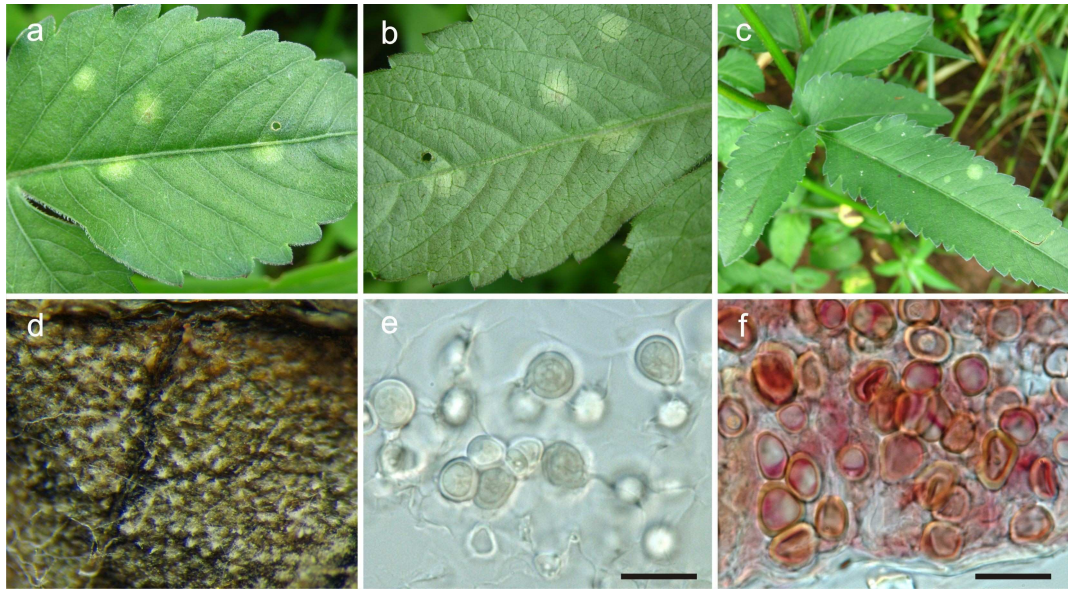


Fig 21 – *Entyloma bidentis*: flat yellow leaf spots typical of attack by *E. bidentis* and distinct from those caused by *E. guaraniticum* (compare with Fig 8) (a-c); hypophyllous sporidial tufts on stomata (d); teliospores (e-f). Bars = 20.0 μm .

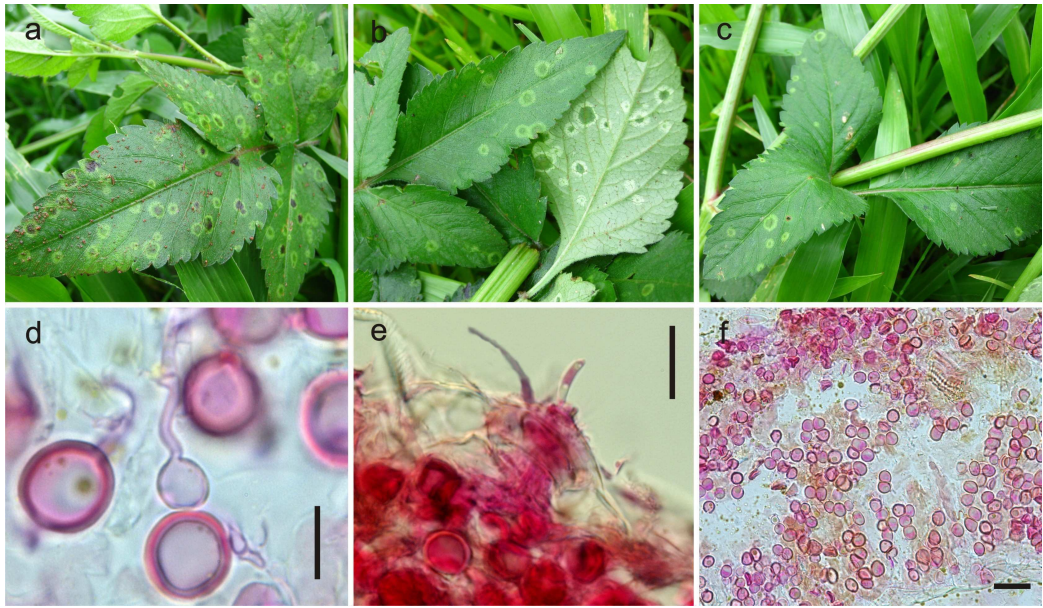


Fig 22 – *Entyloma compositarum* on *Bidens pilosa*: flat yellow leaf spots, with green center and yellow greenish halo adaxially, typical of attack by *E. compositarum* and distinct from those caused by *E. guaraniticum* (compare with Fig 4) (a-c); teliospores germinating inside the host tissue (d). Bar = 10.0 µm; anamorph arising through stoma (e). Bar = 20.0 µm; teliospores not densely packed and embedded in host tissue (f). Bar = 40.0 µm.

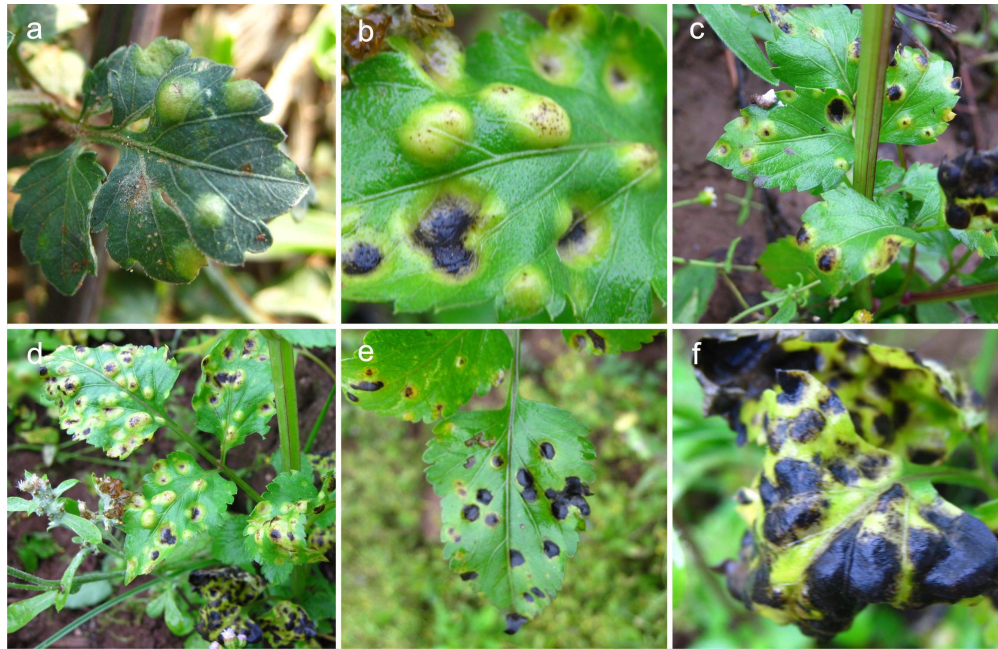


Fig 23 – *Entyloma guaraniticum*: leaves bearing yellow blisters and distortions becoming black and necrotic with age, typical of white smut attack (a-f).

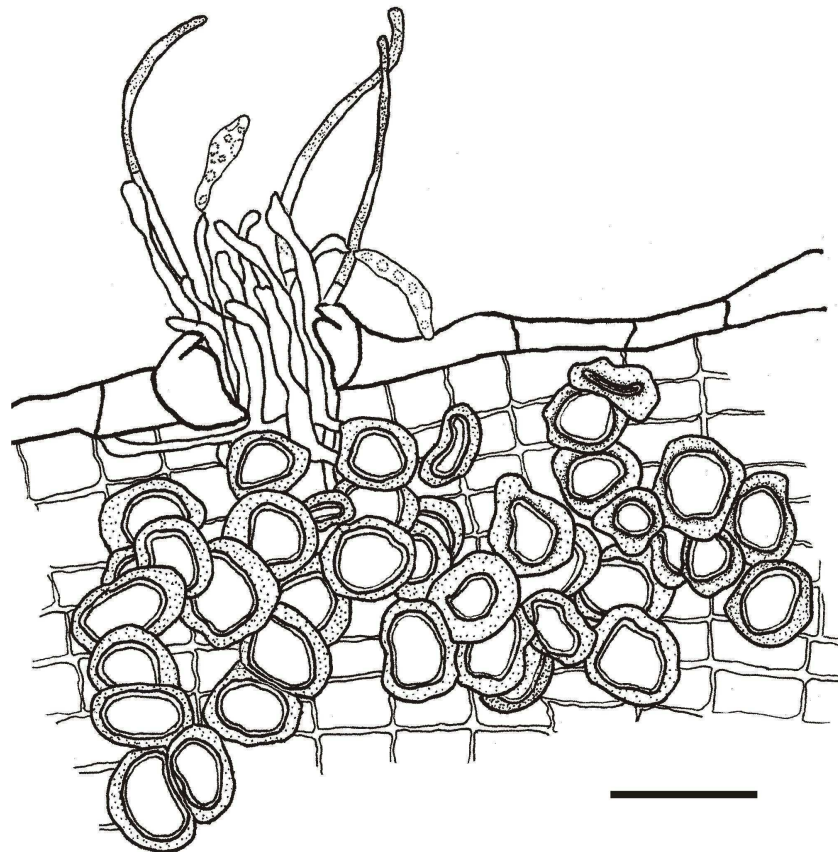


Fig 24 – *Entyloma guaraniticum*: teliospores embedded in host tissue germinating to produce needle-shaped and sickle shaped sporidia through a stoma. Bar. = 20.0 μ m.

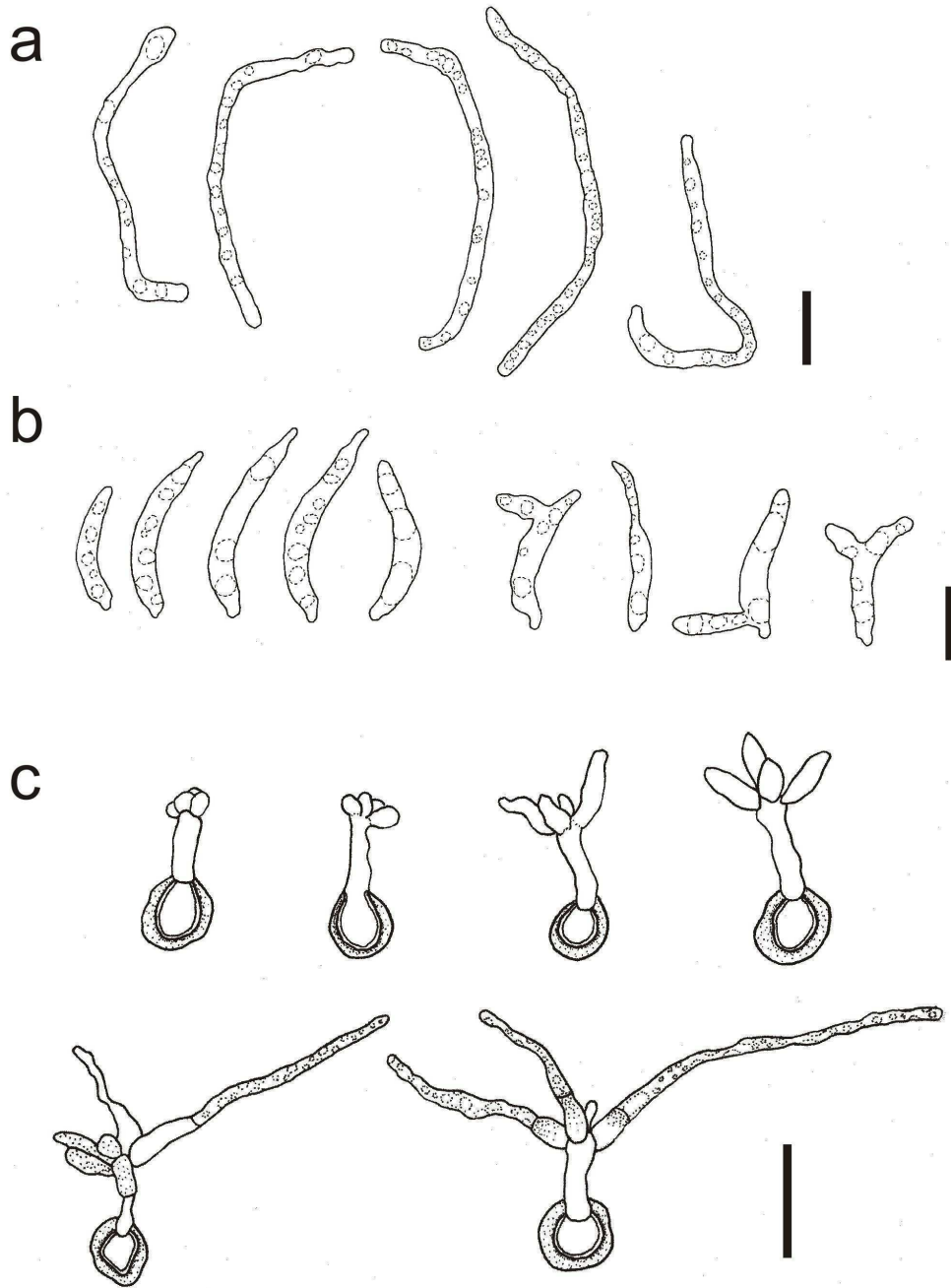


Fig 25 – *Entyloma guaraniticum*: needle-shaped sporidia (a); sickle-shaped sporidia (b); *germinating ustilospores*, 24-36 hours of incubation (c). Bars = 20 μm .

CONCLUSÕES GERAIS

O levantamento e estudo da micobiota fitopatogênica de *Bidens pilosa* (picão-preto), importante espécie daninha de ecossistemas agrícolas no Brasil, com centro de origem na América Tropical, levou à descoberta de dez taxa fúngicos sendo seis representando novas associações com este hospedeiro no Brasil: *Cercospora maculicola*, *Entyloma compositarum*, *Entyloma guaraniticum*, *Golovinomyces cichoracearum* var. *cichoracearum*, *Plasmopara halstedii* e *Podosphaera fusca*. Foi comprovada a patogenicidade de *Cercospora bidentis* e *Cercospora maculicola* a *Bidens pilosa*. Neste estágio do trabalho nenhum dos fungos encontrados associados a *B. pilosa* aparenta ainda ter claro potencial para o uso em programas de controle biológico inundativo.

No levantamento e estudo da micobiota fitopatogênica de *Bidens subalternans* (picão-amarelo), foram encontrados doze taxa fúngicos sendo que um representa uma nova espécie para ciência (*Pseudocercospora* **sp.nov.**) e nove representam novas associações com este hospedeiro no Brasil: *Cercospora bidentis*, *Cercospora maculicola*, *Colletotrichum* sp., *Entyloma bidentis*, *Entyloma guaraniticum*, *Plasmopara halstedii*, *Podosphaera fusca*, *Sphaceloma bidentis*, e *Uromyces bidentis*.

Comprovou-se a patogenicidade de *Cercospora bidentis*, *Cercospora maculicola*, *Colletotrichum* sp. e *Sphaceloma bidentis* a *Bidens subalternans*. As espécies *Colletotrichum* sp. e *Sphaceloma bidentis* destacaram-se como tendo aparente potencial como agentes de biocontrole inundativo para as duas espécies de plantas-alvo aqui estudadas. Estes dois fungos crescem em meio de cultura e ao

serem inoculados massalmente sobre plantas das duas espécies produzem níveis severos de doença que podem levar as plantas à morte.